A Review of Depleted Uranium Biological Effects: *In vitro* studies

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Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
NOV 2010		**00-00-2010 to 00-00-2010**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
A Review of Depleted Uranium Biological Effects: In vitro studies	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Uniformed Services University,Armed Forces Radiobiology Research Institute,8901 Wisconsin Avenue,Bethesda,MD,20889-5603**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
Presented at the Depleted Uranium Symposium, held November 4, 2010, at the Armed Forces Radiobiology Research Institute

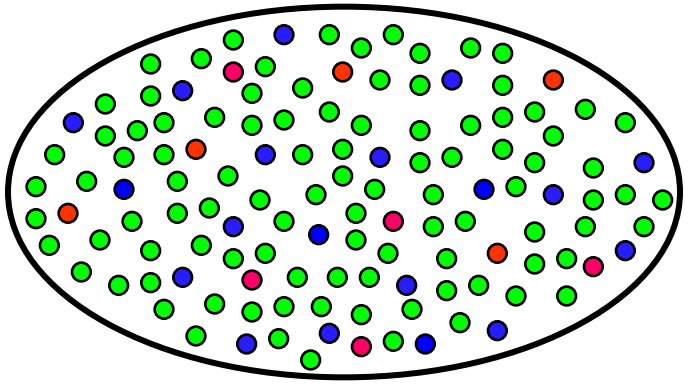
14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**60**	

What is "Depleted Uranium"

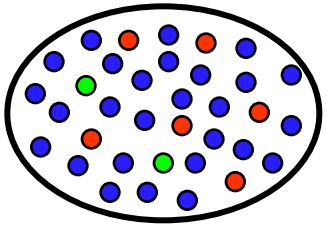
Natural Uranium

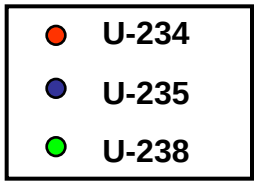


Enrichment process

Enriched Uranium

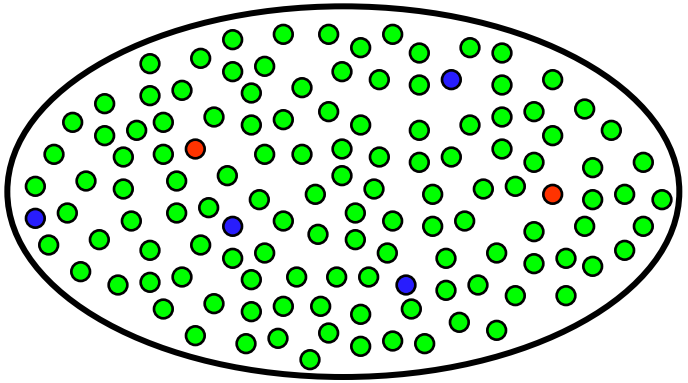
Increased ratio of U-234/U-235 to U-238





Depleted Uranium

Decreased ratio of U-234/U-235 to U-238

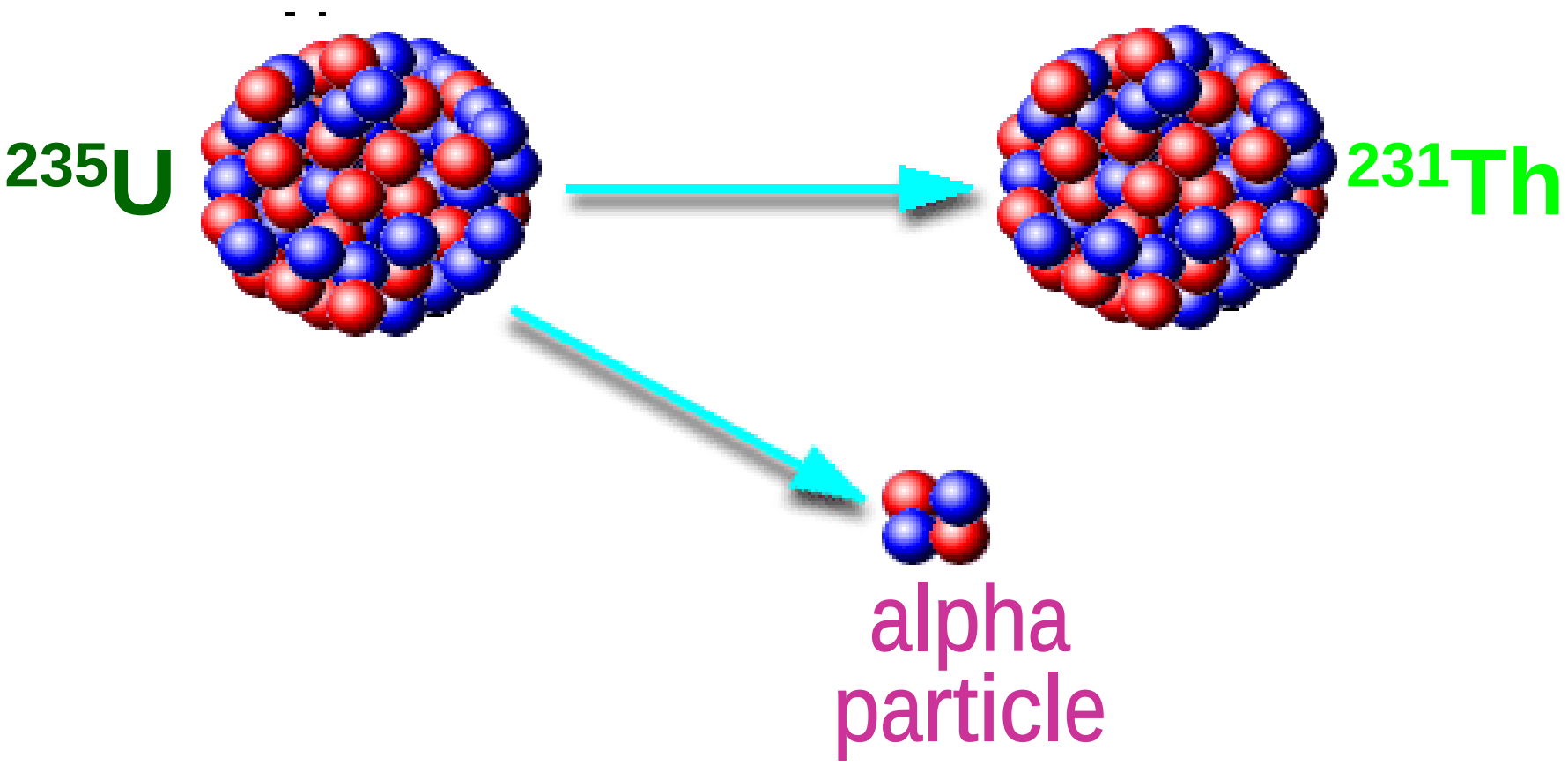


Reduced U^{234}, no daughter products, radium, radon

DU is Radioactive:

Alpha Particle Emitter

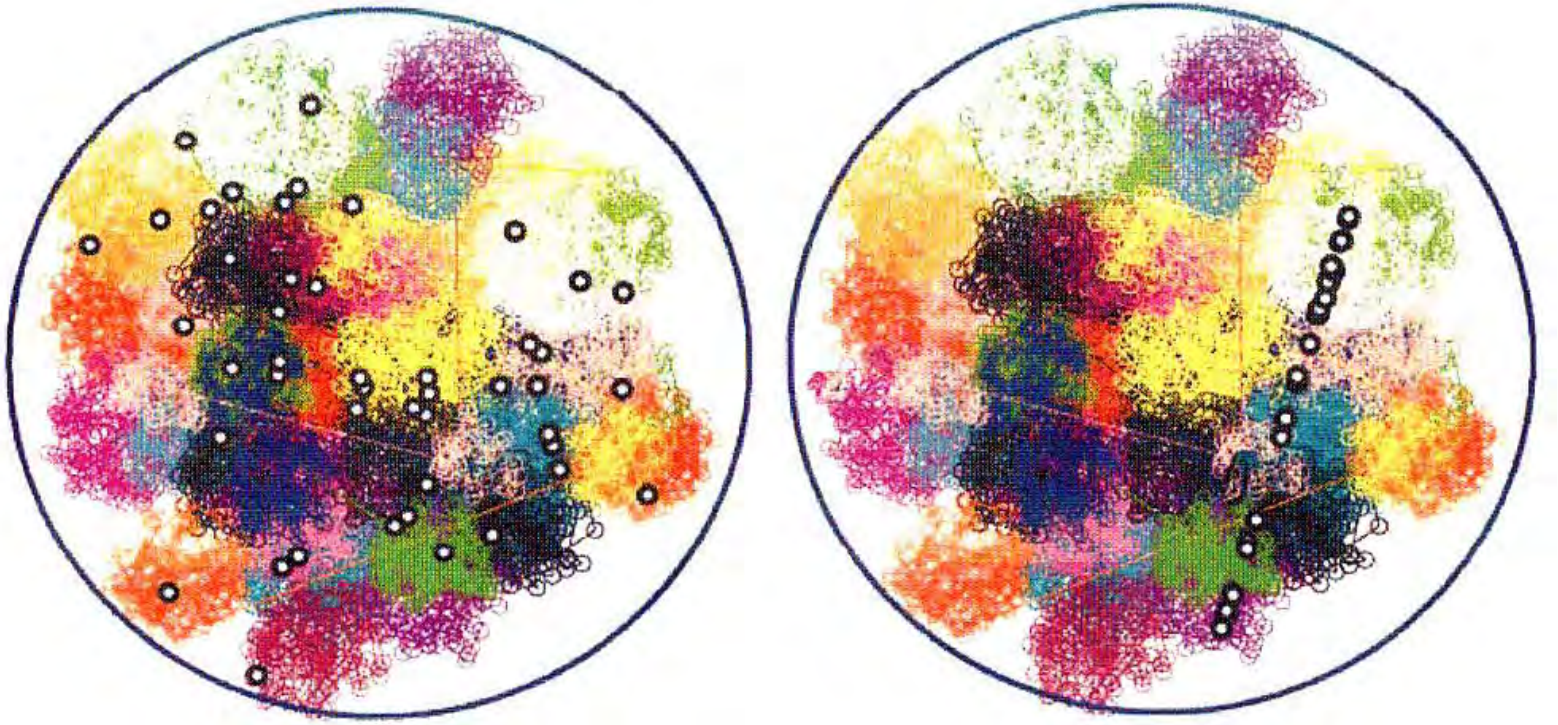
Spontaneous emission of particles from unstable nuclei



Alpha particles are more hazardous than x rays or gamma rays, because they deposit their energy over very small distances

X rays

Alpha particles



o = chromosome break

Comparison of the Relative Contribution of Uranium Isotopes*

(natural and depleted)

Isotope	Specific Activity (μCi/g)	DU SA by WT% (μCi/g)	Natural Uranium SA by WT% (μCi/g)
^{238}U	0.333	0.332	0.331
^{236}U (not naturally occurring)	63.6	0.0001	0
^{235}U	2.2	0.0044	0.051
^{234}U	6200	0.093	0.310
Total		**0.4295**	**0.692**

*Contribution of the daughter products is not included.

Questions Regarding DU And Its Health and Biological Effects that Prompted Our Research

1) **Is long-term exposure to internalized DU carcinogenic?**

2) **Does DU cause radiation effects?**

3) **Does DU cause transgenerational effects?**

4) **Can we distinguish between DU and other exposures (radiation, chemical)?**

Research Approach:
Follow Regulatory Agency Approach

IARC,NTP, FDA, EPA

Carcinogenic Hazard Evaluation

Transformation + Mutagenicity + Cytogenicity

Animal Carcinogenesis Model

Human Epidemiology

Limitations of Using DU Compounds *in vitro*

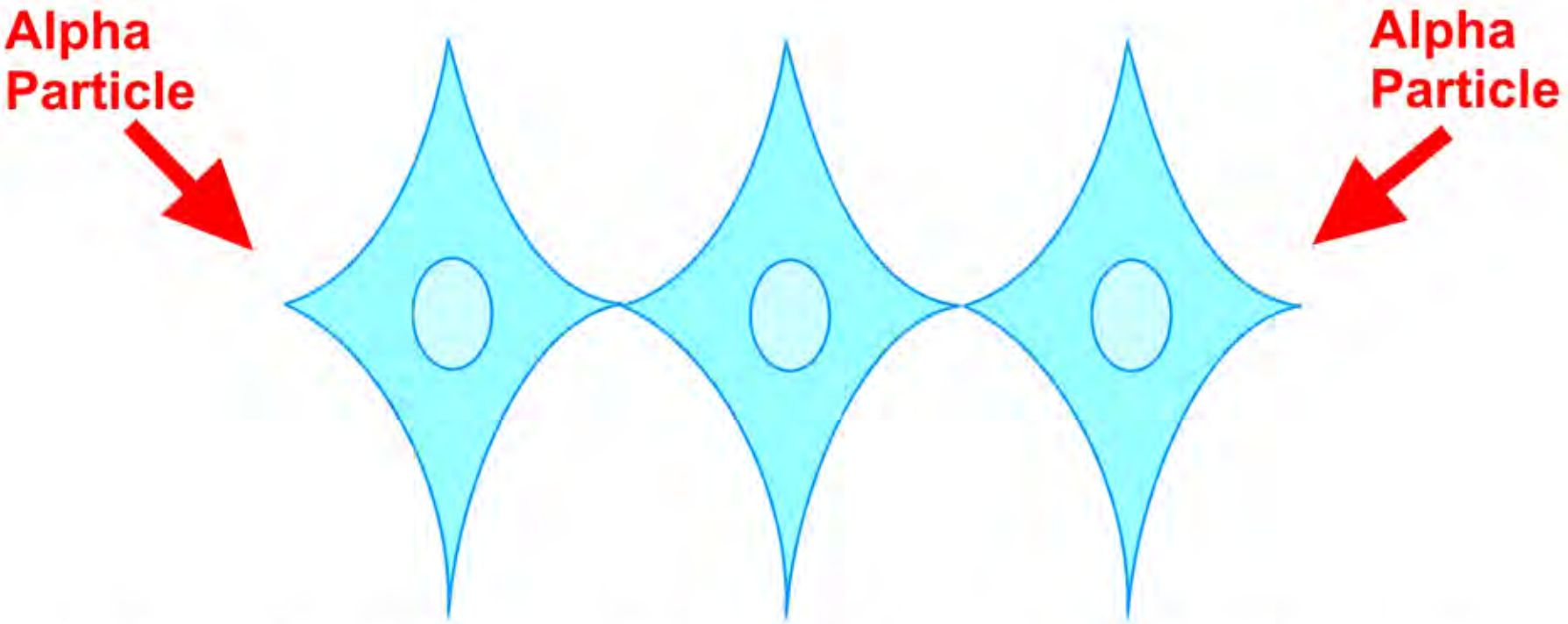
1) **DU- uranyl nitrate, uranyl chloride, acetate (soluble)**

2) **DU- uranium dioxide (insoluble)**

3) **Uptake of uranium by cells**

4) **Radiation dose to cells**

Radiation Dose Measurement "Microdosimetry"



≤ 1% cells hit; approximately 18 cGy (soluble DU)

≤ 7% cells hit; approximately 22 cGy (insoluble DU)

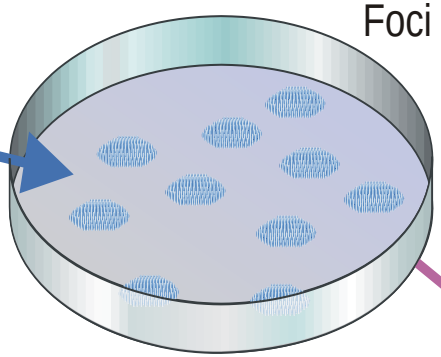
≤ 21% cells hit; approximately 24 cGy (insoluble DU)

What Endpoints do we Measure?

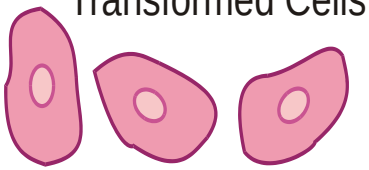
+ Exposure

Neoplastic Transformation

Normal Cells



Foci of Transformation

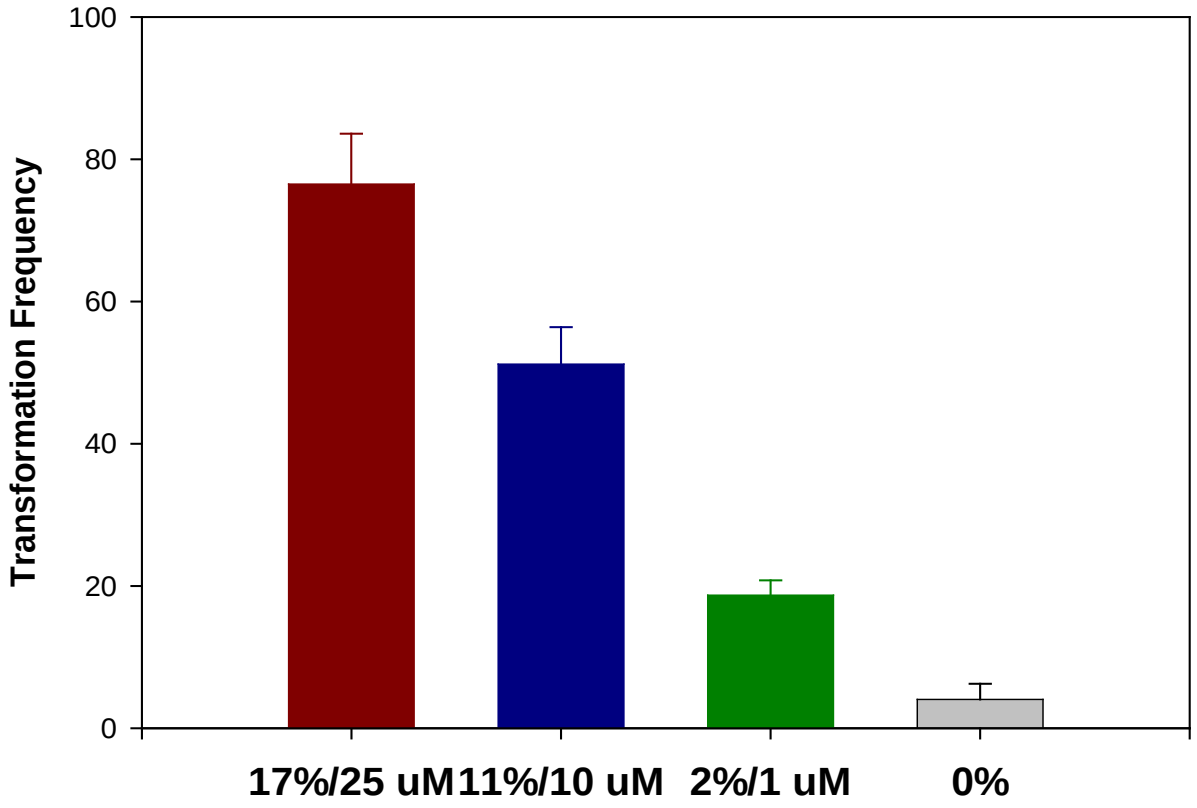


Transformed Cells

Cause Hard Tumors in Mice

Transformation to Tumorigenic Phenotype

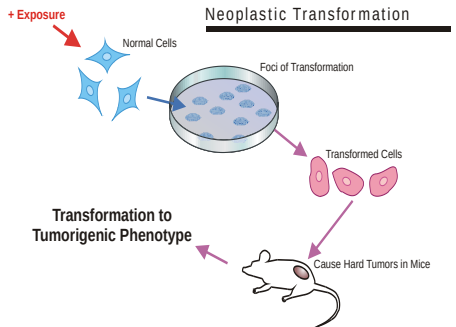
Comparison of Traversals of Nucleus by Alpha Particles and DU Concentration

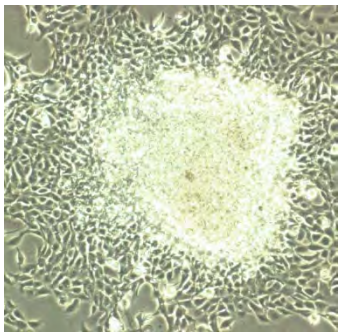


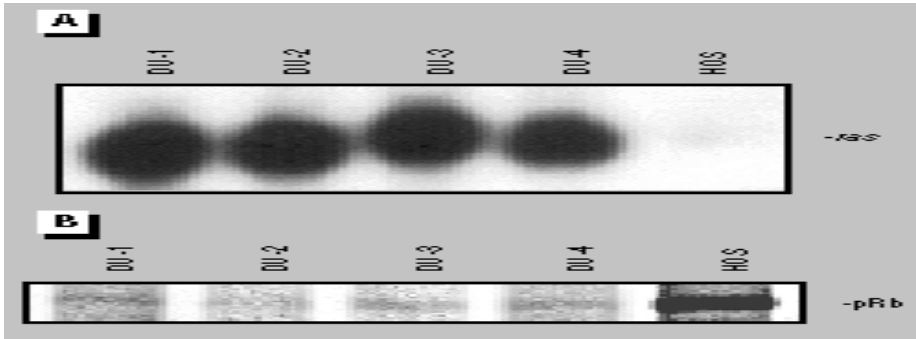
Heavy-Metal Induced Transformation of Human Osteoblast Cells

	Untreated	Ta (soluble)	DU (soluble) (1%)	DU (insoluble) (7%)	DU (insoluble) (21%)	HMTA (insoluble)	NI (insoluble)	PbCr (IV) (Insoluble)	Alpha Particles 5 cGy, 120 keV (100%)
Transformation Frequency (per survivor x 10^{-4})	4.4 ± 1.1	5.2 ± 1.0	49.6 ± 4.8	81.2 ± 6.1	269.5 ± 33	37.6 ± 5.1	46.5 ± 4.9	38.2 ± 4.0	68.2 ± 4.0
Morphology	Flat	Flat	Transformed	Transformed	Transformed	Transformed	Transformed	Transformed	Transformed
Saturation Density (x 10^5 cells)	2.6	2.2	6.9	6.6	7.1	6.1	7.3	5.9	5.9
Soft-agar colony Formation (PE%)	2	2	47	61	78	52	49	37	37
Tumorigenicity (Mice w/tumors Per Mice w/o)	0/240	0/12	22/47	33/50	15/20	8/20	5/12	4/12	4/12

Miller, *et al, Environmental Health Perspectives*, Vol. 1998; Miller, *et al, Carcinogenesis*, Vol. 22, 2001.







Neoplastic Transformation Studies (Multiple Laboratories)

1) **Human osteoblast cells (bone) – HOS (Miller et al 1998; Miller et al 2001, Miller et al, 2003; Miller et al 2005)**

2) **Human bronchial cells (lung) – BEP2D (Xie et al, 2010)**

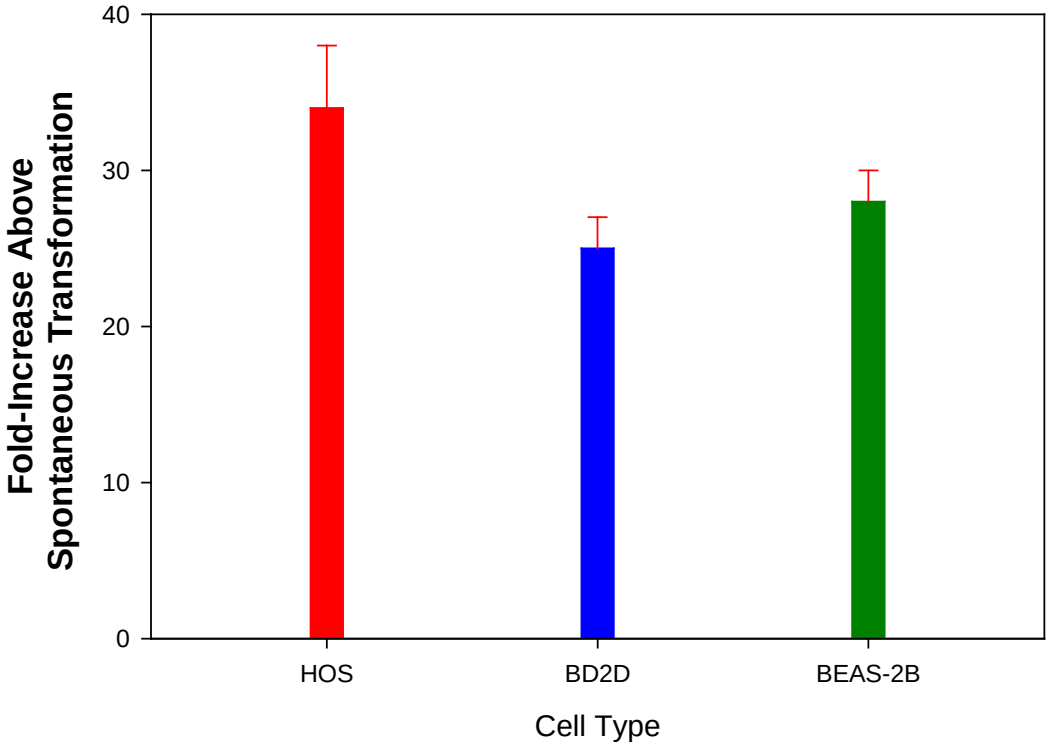
3) **Human bronchial cells -BEAS2B (Yang et al, 2010)**

Malignant Transformation by Insoluble DU Compounds

1) **Human osteoblast cells (bone) – HOS (Miller et al 1998; Miller et al 2001, Miller et al, 2003; Miller et al 2005)**

2) **Human bronchial cells (lung) – BEP2D (Xie et al, 2010)**

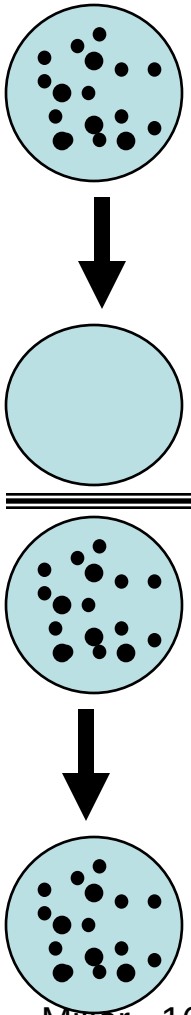
3) **Human bronchial cells - BEAS2B (Yang et al, 2010)**



Mutagenesis Studies

HPRT Gene ASSAY

The Hypoxanthine Phosphoribsyltransferase Assay.



HPRT +/+

6-thioguanine = Poison

HPRT -/-

6-thioguanine = OK

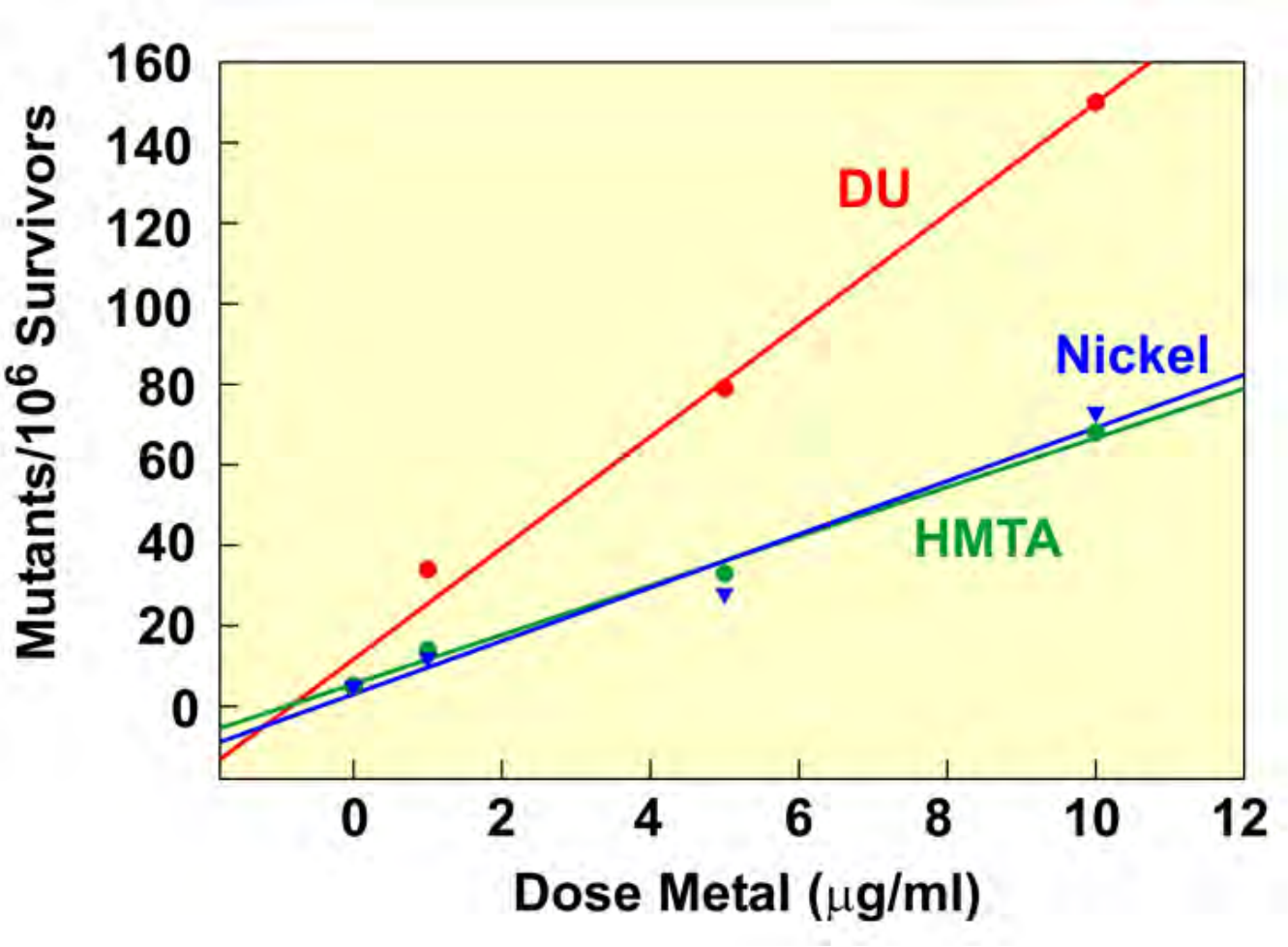
So, if cell acquire mutation in HPRT, it become resistant

to 6-thioguanine compound

We can directly count mutant colonies and compare this number with number of cell seeded on plate

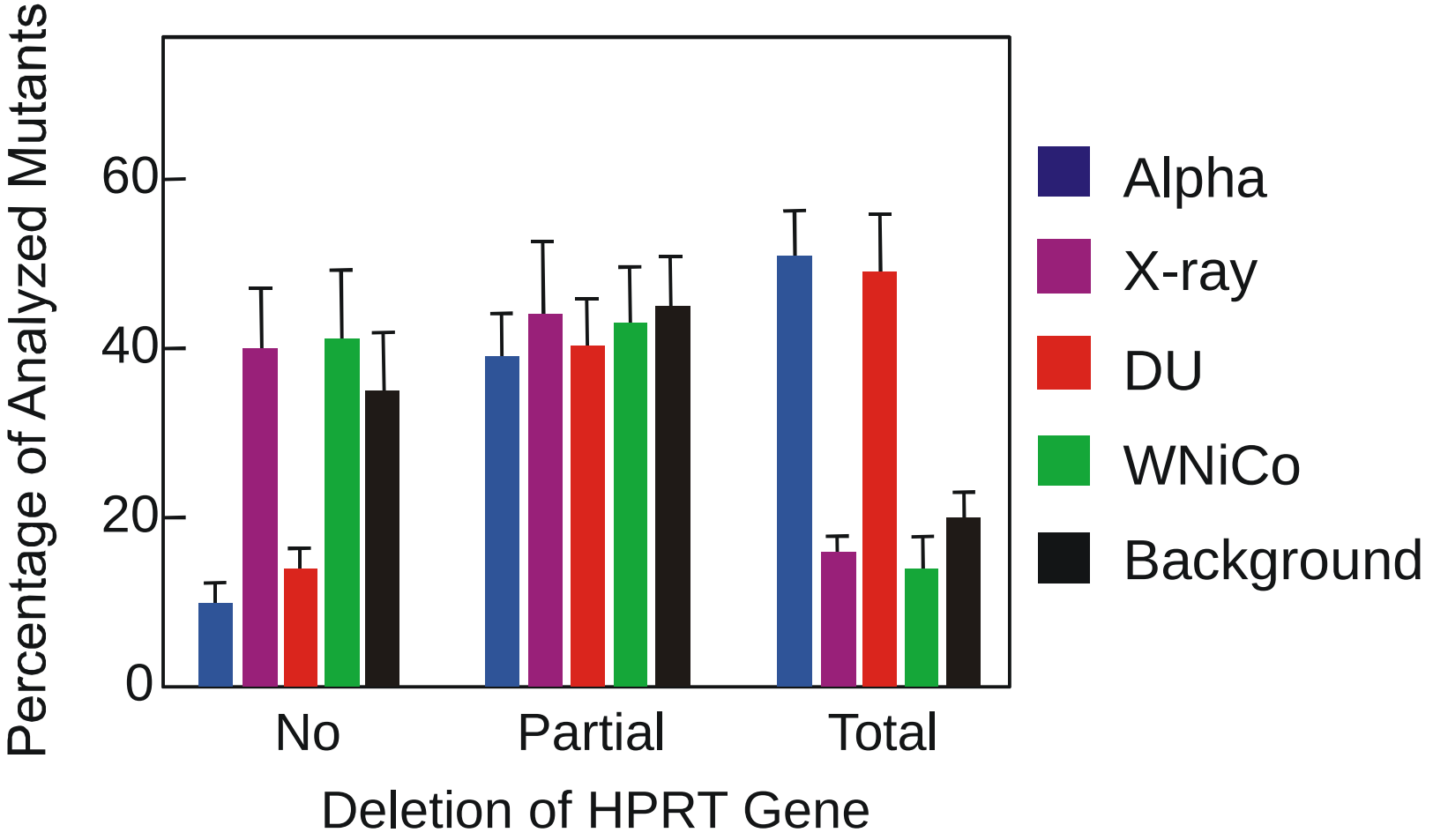
DU – Uranium Dioxide Mutagenicity

HPRT Mutations in V79 Cells



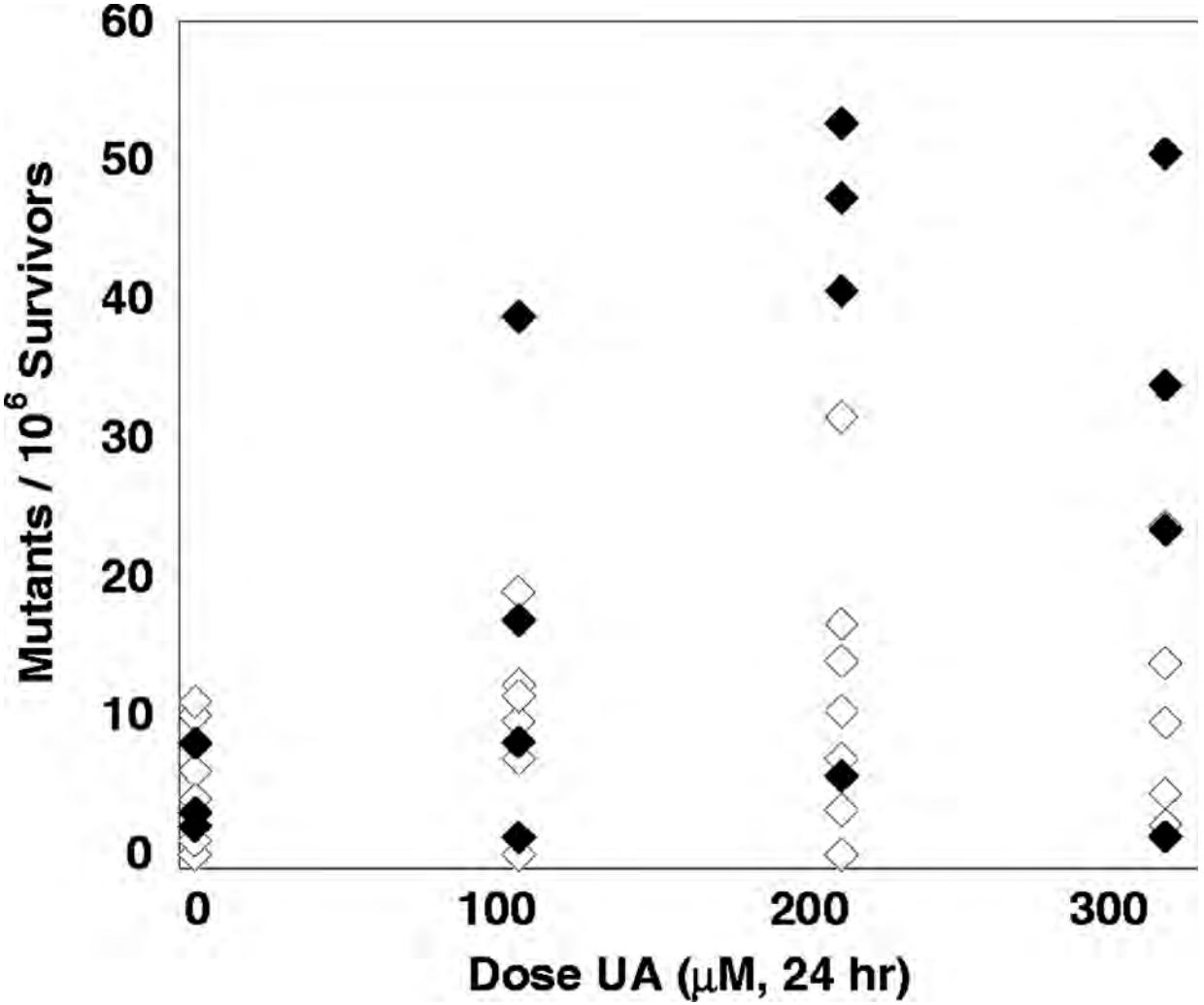
Unpublished data

Mutation Spectrum of HPRT Gene In V79 Cells



Mutagenicity of DU-Uranyl Acetate – Stearns Laboratory NAU

6-Thioguanine-resistant cells obtained after 24 h exposure of CHO-AA8 (open diamonds) and CHO-EM9 (closed diamonds) cells to UA.

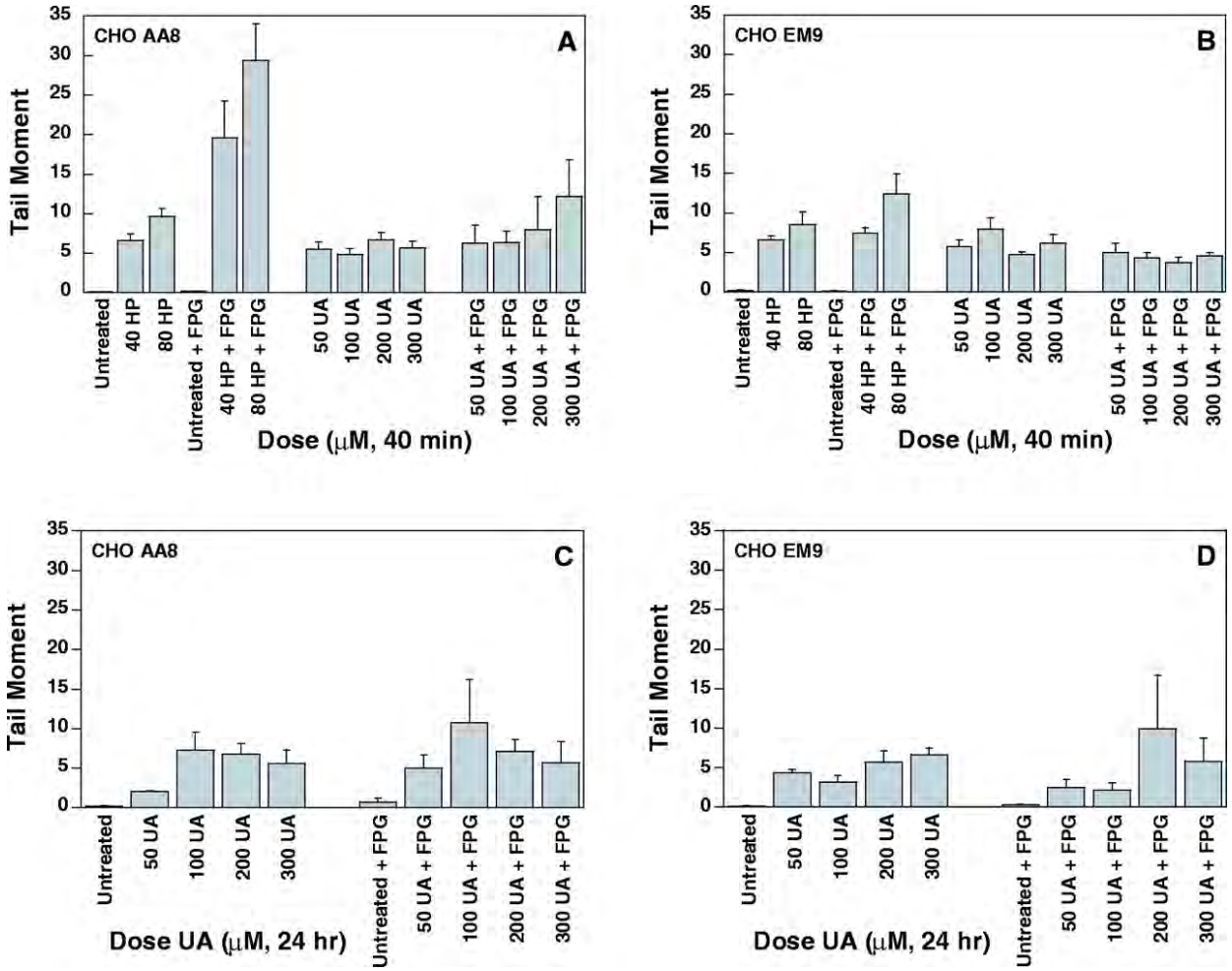


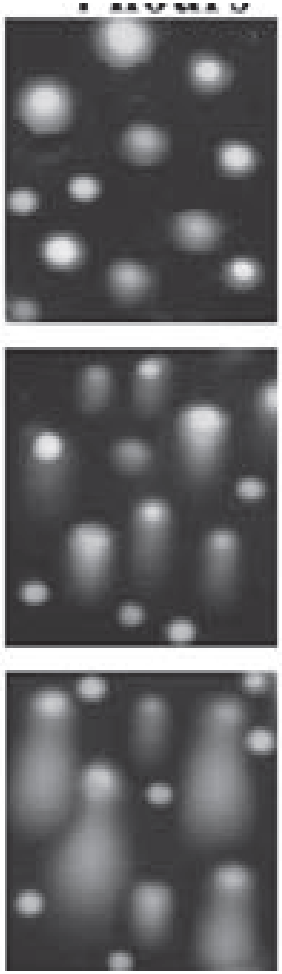
Stearns D M et al. Mutagenesis 2005;20:417-423

DNA Damage Studies

DNA Damage Induced by DU – Stearns Laboratory NAU

Analysis of DNA damage induced by UA and H2O2 by the comet assay.

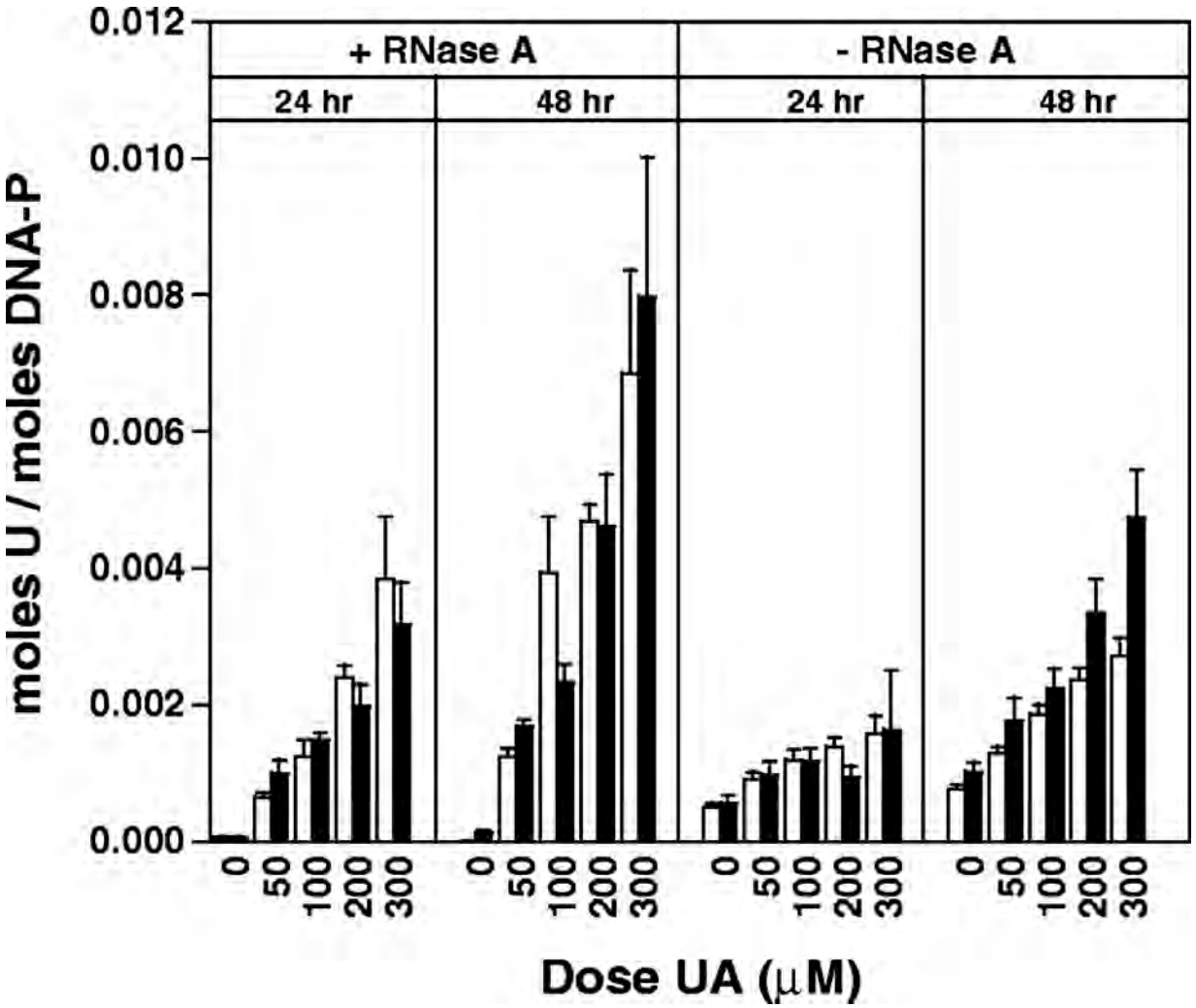




Stearns D M et al. Mutagenesis 2005;20:417-423

DU Binds to DNA

Measurement of uranium–DNA binding in CHO AA8 (open bars) versus CHO EM9 (closed bars) cells exposed to UA for 24 or 48 h by ICP-OES.

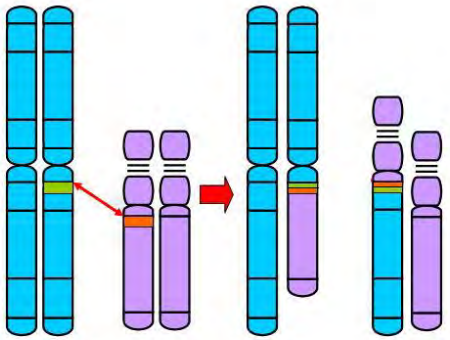


Stearns D M et al. Mutagenesis 2005;20:417-423

Genotoxicity Studies

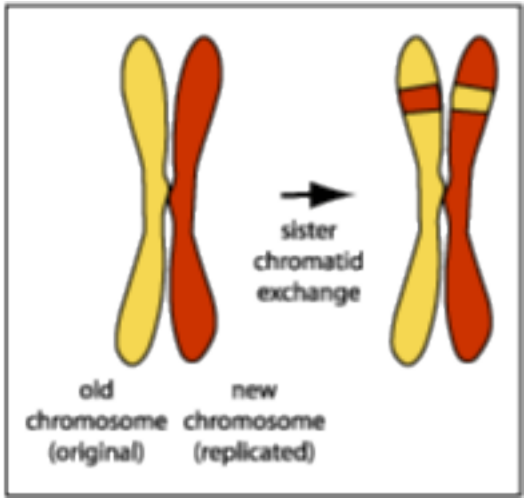
Earliest studies with DU in vitro:
Lin et al*, Mutation research* 1993, Cytogenetic toxicity of uranyl nitrate in Chinese hamster ovary cells.
Results:
DU induced sister chromatid exchanges (SCEs)

Chromosomal Changes

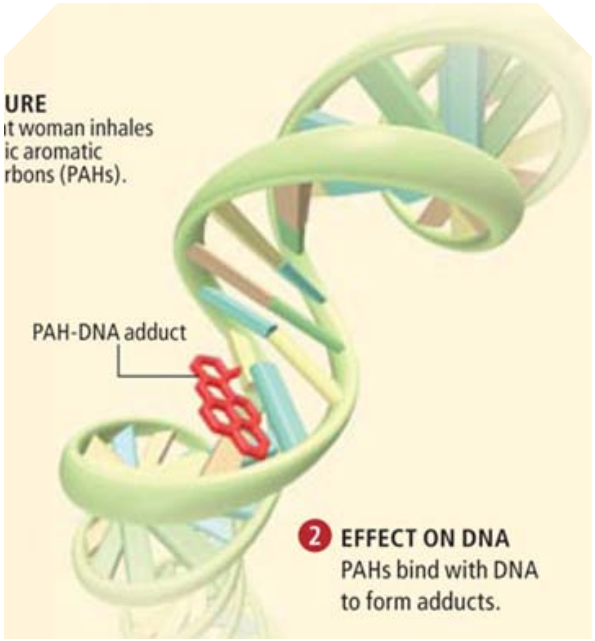


Translocations

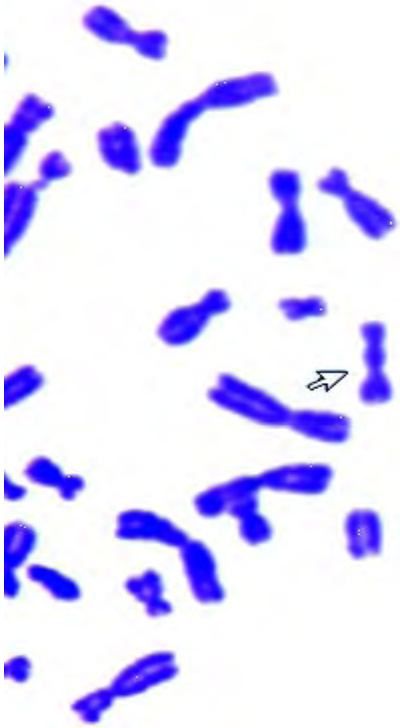
Sister Chromatid Exchange



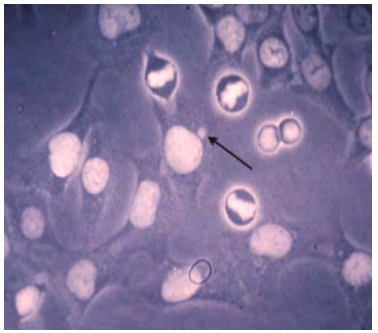
DNA Adducts



Dicentrics



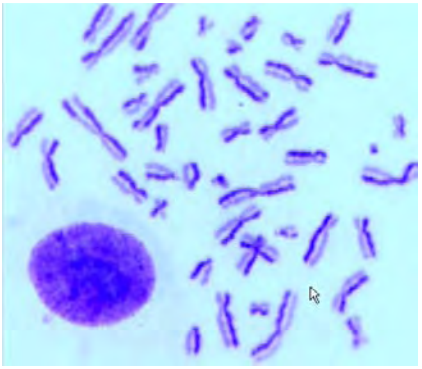
Micronuclei

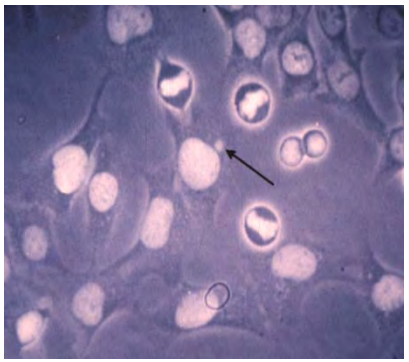


Short-term Assays: DNA or Chromosome Endpoints

Mutagenicity: **Gene Mutation**
Cytogenicity: **Chromosomal Damage**
Genomic Instability: **Chromosomal damage (clonal descendents)**

HPRT Gene





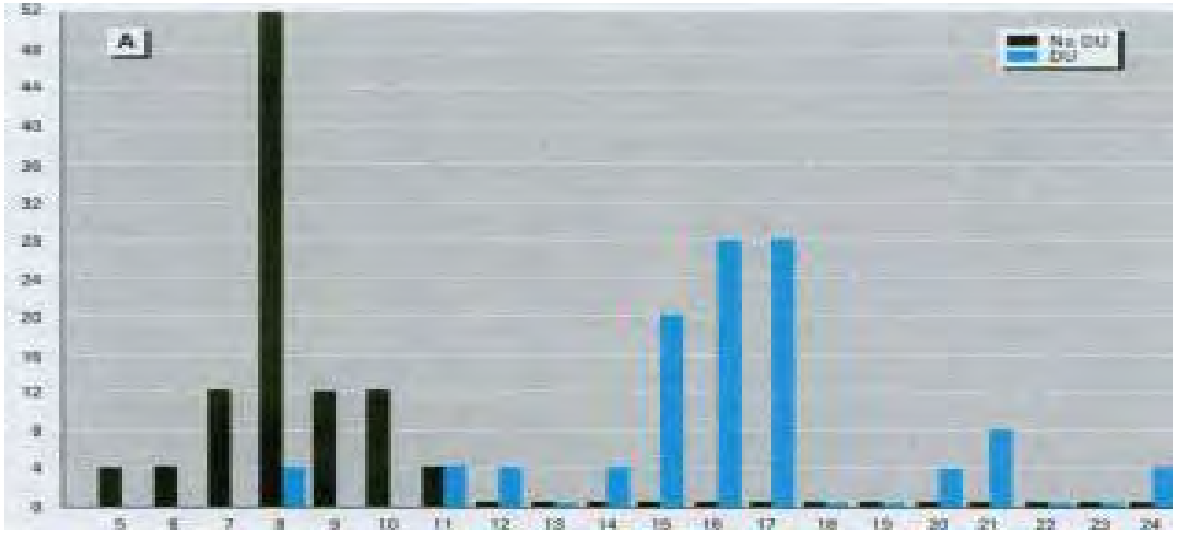
Heavy Metal Genotoxicity

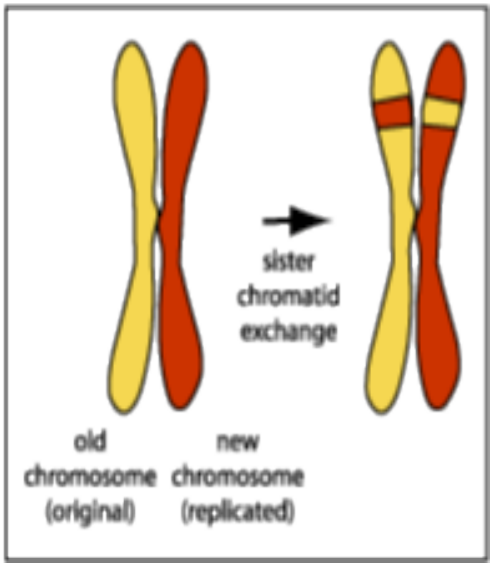
	DU (Soluble)	DU (Insoluble)	rWNiCo	Be	Ni
Micronuclei Induction	↑	↑	↑	↑	↑
Sister Chromatid Exchange	↑	↑	↑	↑	↑
DNA Filter Elution (DNA strand break)	↑	↑	↑	↑	↑
Dicentric Formation	↑	↑	no change	ND	no change

Miller *et al, Carcinogenesis*, Vol 22, 2001. Miller *et al., Metal Ions in Biology and Medicine,* Vol 6, 2001.

Induction of Chromosomal Damage

Frequency of Cells with SCE (%)



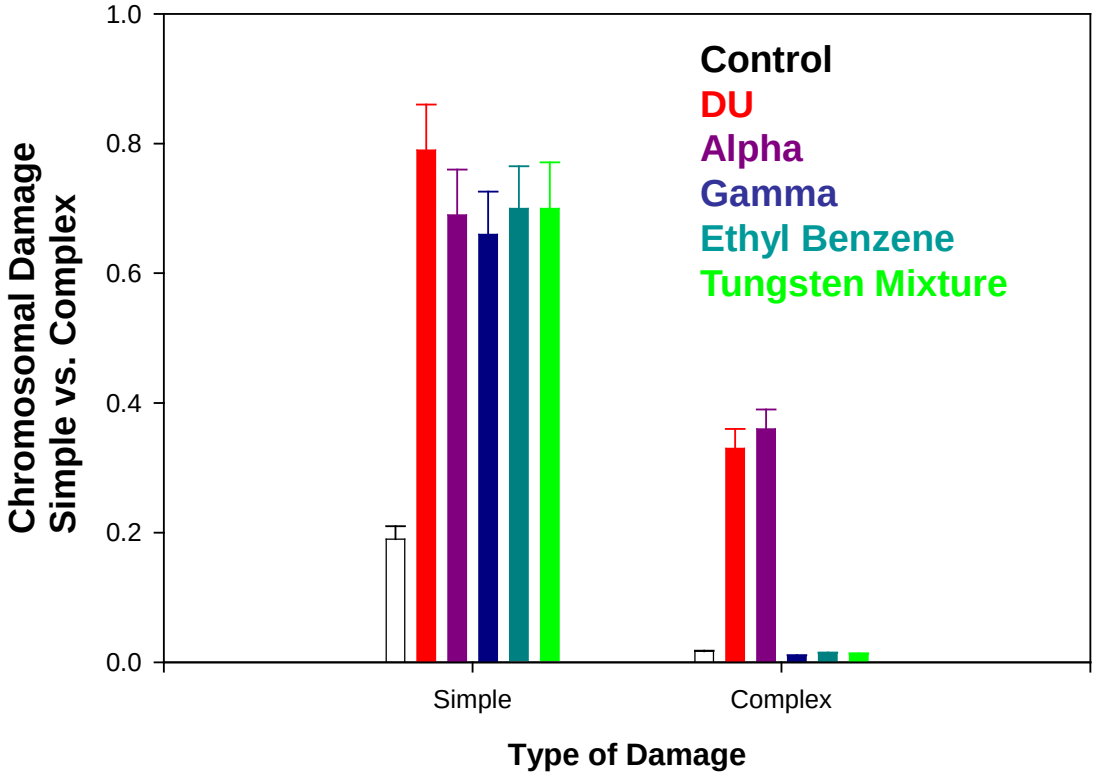


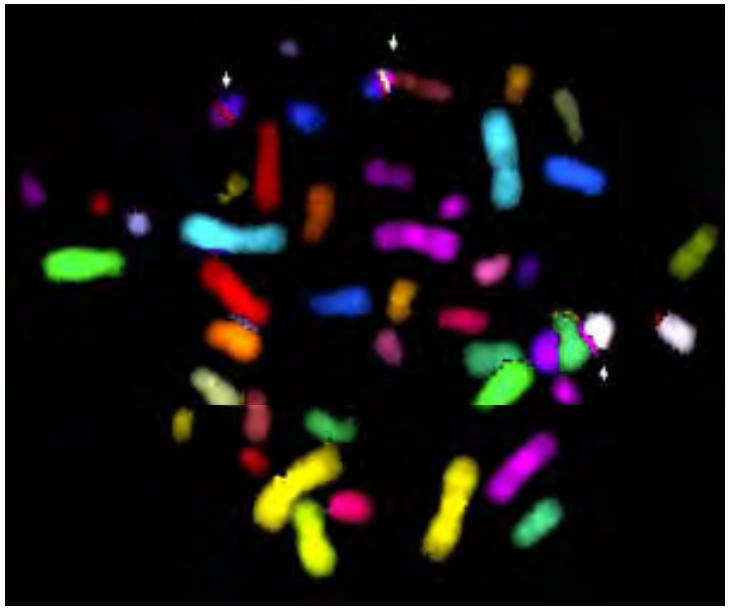
Number of SCE's Per Cell

Mechanisms:
inhibition of DNA replication
direct damage of chromosomes

Miller *et al,* 1998, *Environmental Health Perspectives*

Chromosomal Damage in Human Osteoblast Cells Exposed to DU, Alpha Particles, Gamma Radiation, Tungsten Mixture, Ethyl Benzene *in vitro*



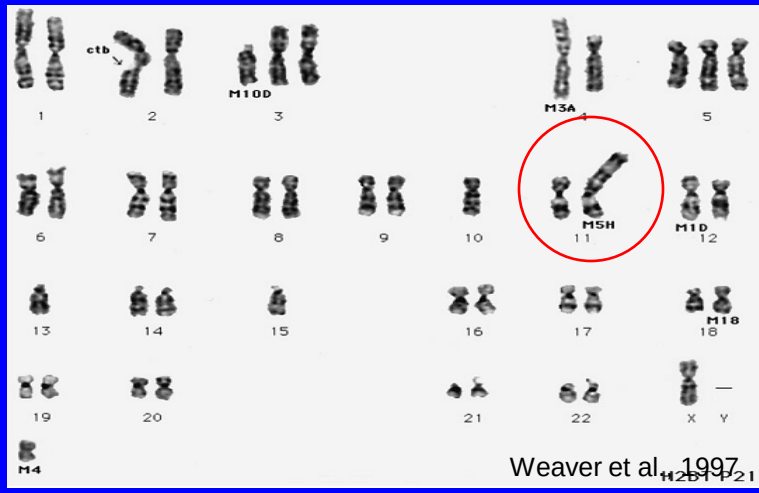


Rona Anderson, MRC
and Patrick Lavelle, Univ of Paris

Chromosome Instability (CIN)

- Hallmark of lung cancer
- Proposed as an early event in carcinogenesis
- 70-80% of lung tumors exhibit CIN
- Complex phenotypes
  - Structural abnormalities
  - Numerical abnormalities

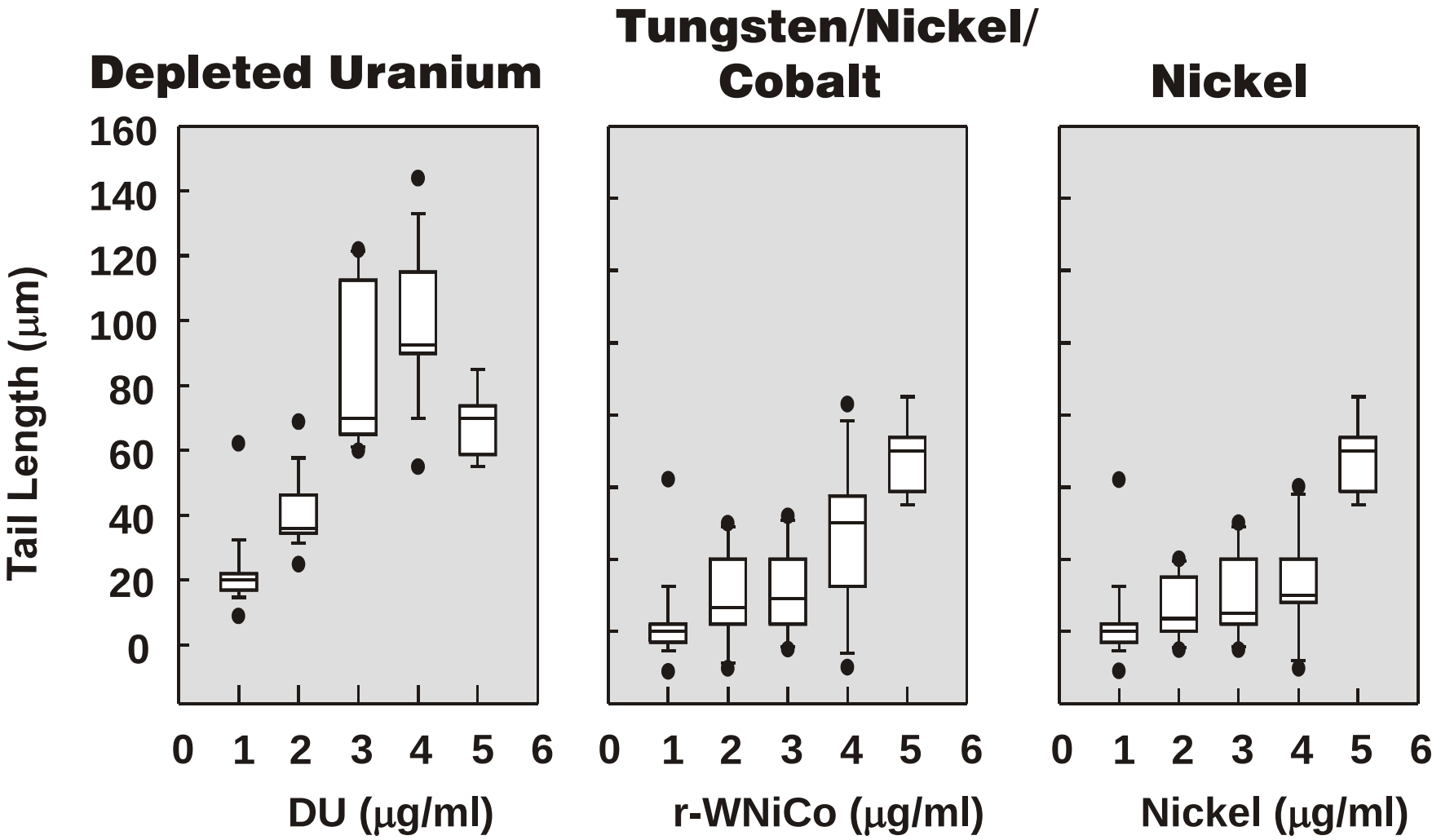
BEP2D Karyotype



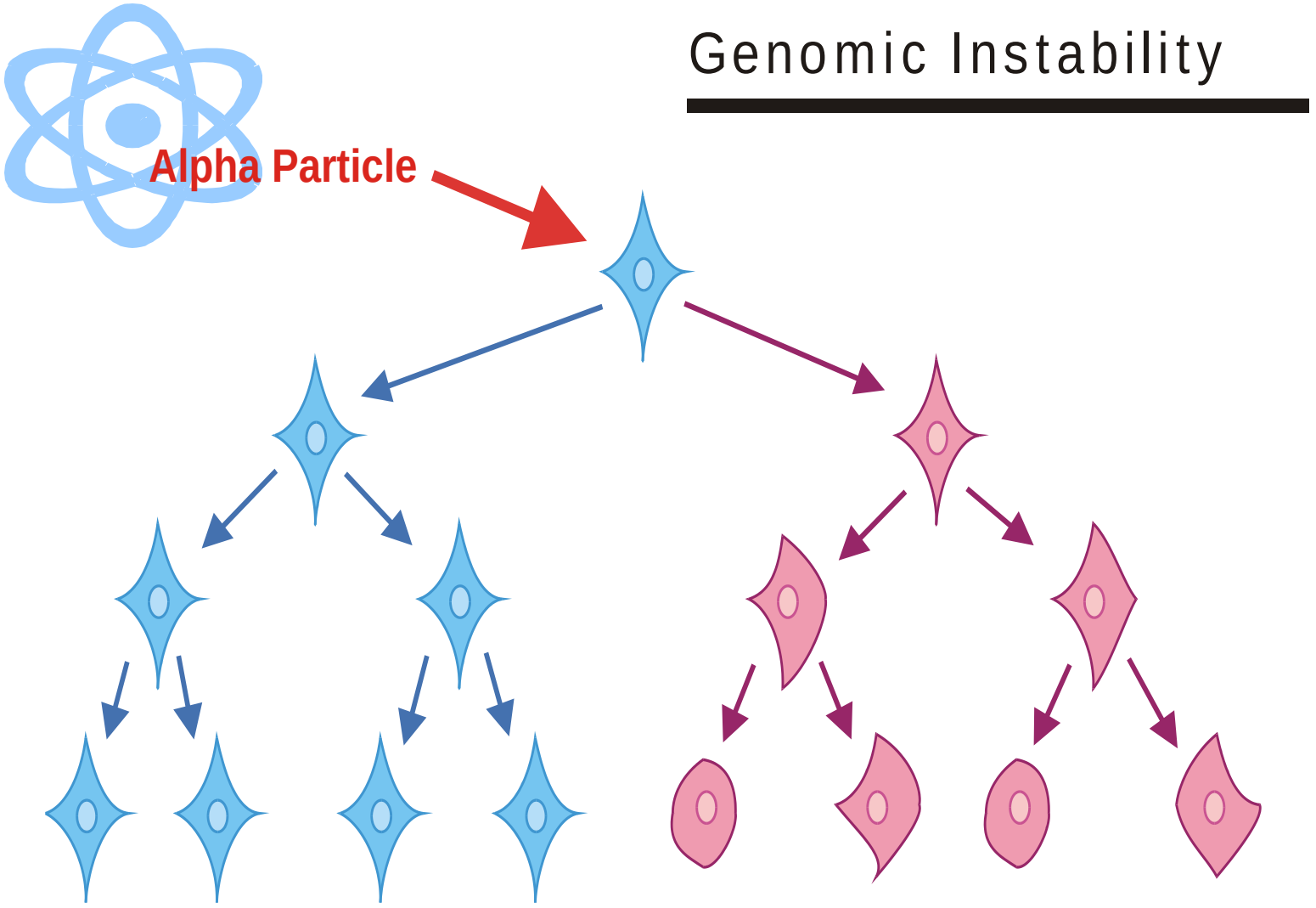
Tumorigenic Karyotype

Genotoxicity

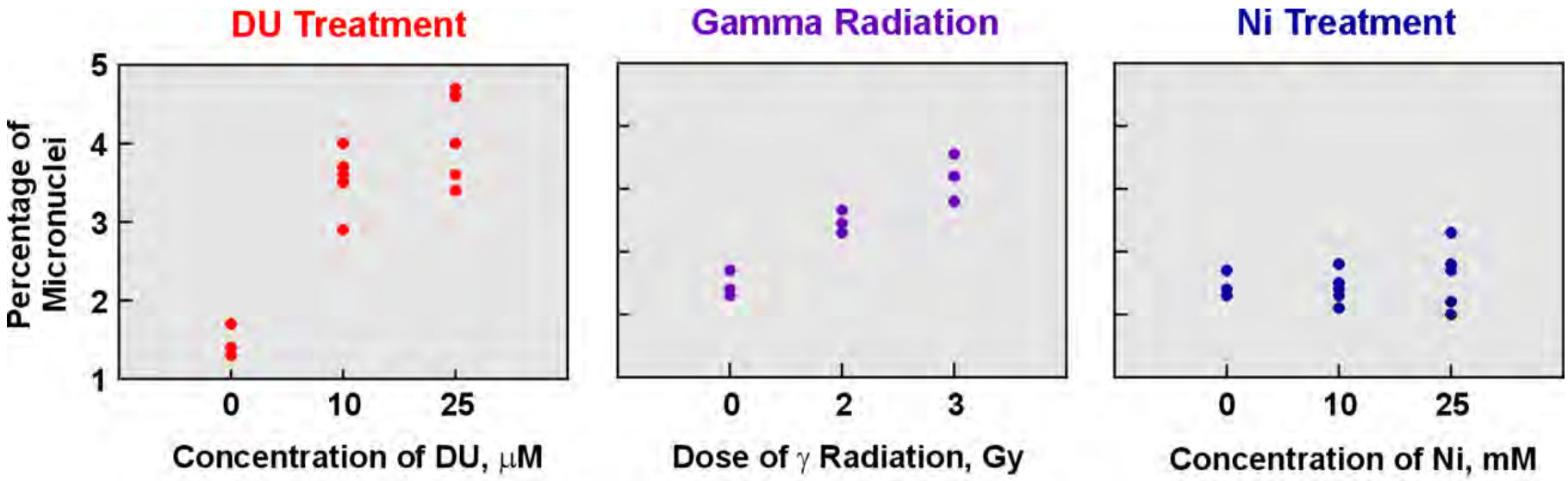
Single Cell Gel Electrophoresis Assay - "Comet" Assay



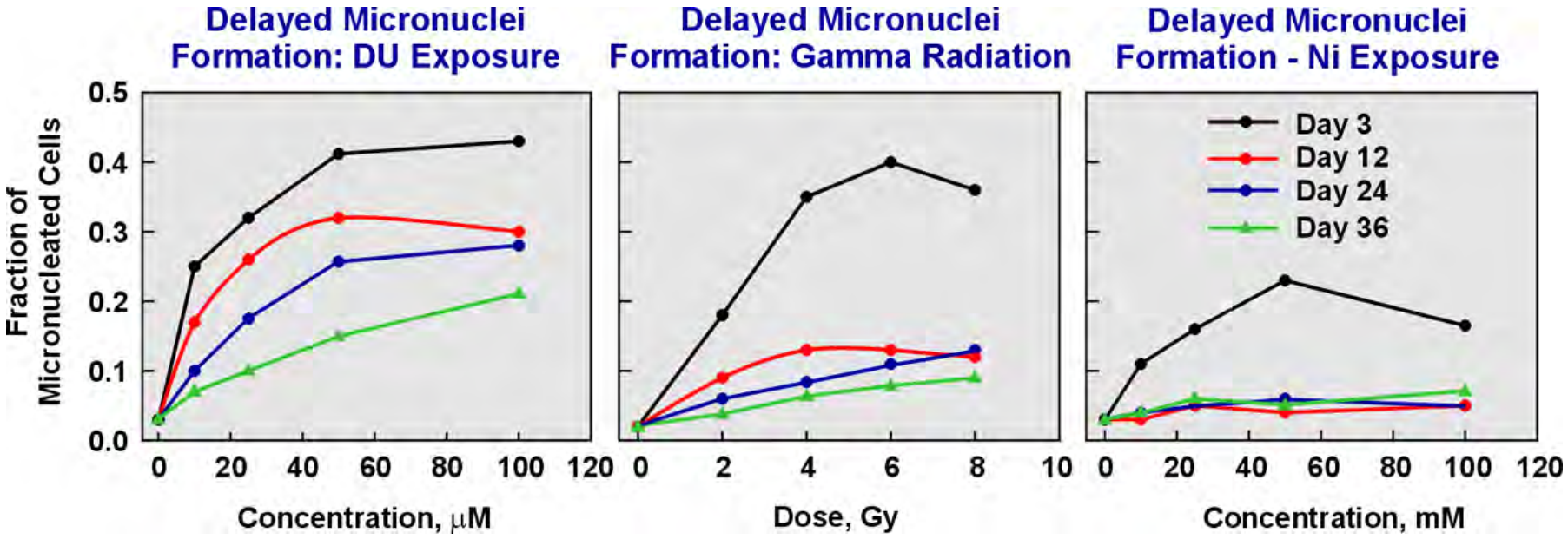
Genomic Instability



Induction of Genomic Instability

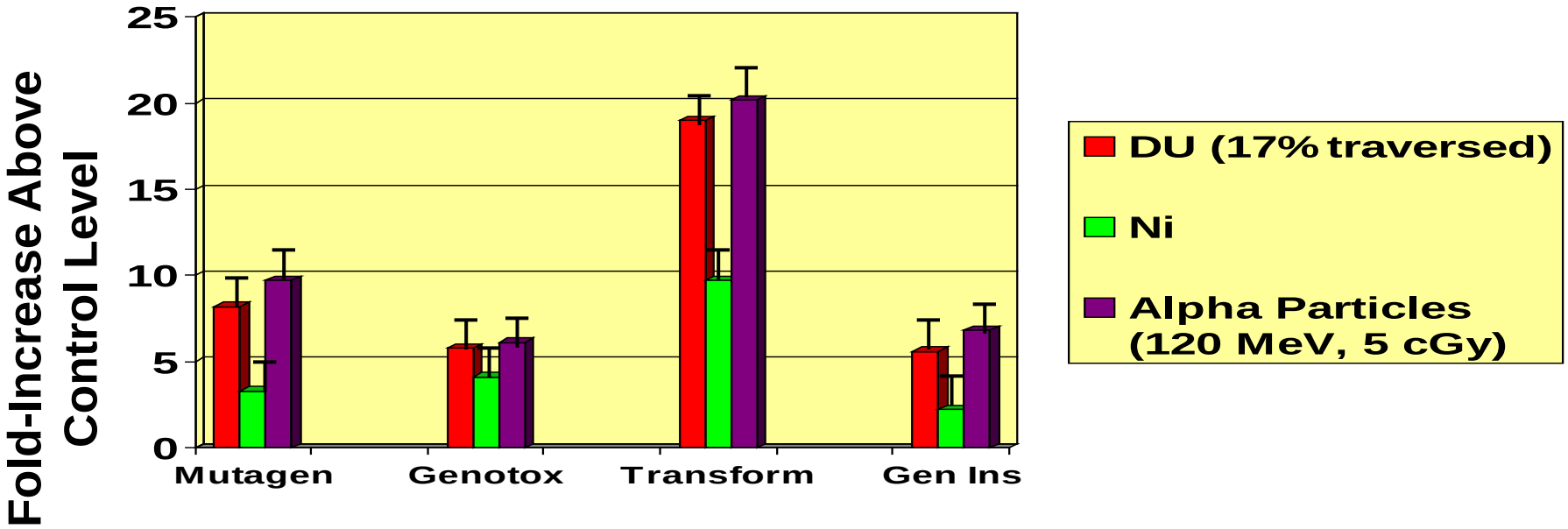


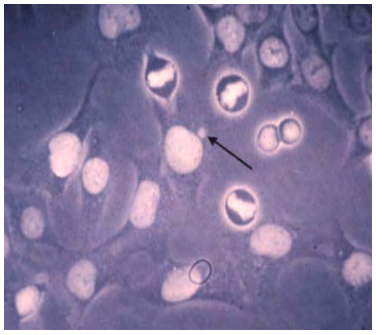
Miller *et al, Journal of Environmental Radioactivity*, 2003;64(2-3):247-59.

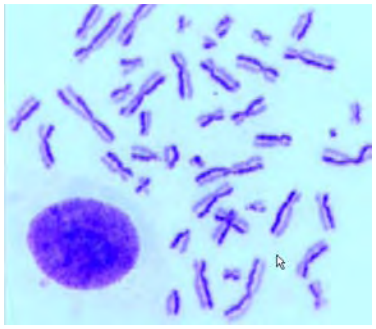

Delayed Micronuclei Formation: DU Exposure
Delayed Micronuclei Formation: Gamma Radiation
Delayed Micronuclei Formation - Ni Exposure
Fraction of Micronucleated Cells
0.0
0.1
0.2
0.3
0.4
0.5
0 20 40 60 80 100 120
Concentration, μM
0 2 4 6 8 10
Dose, Gy
0 20 40 60 80 100 120
Concentration, mM
Day 3
Day 12
Day 24
Day 36

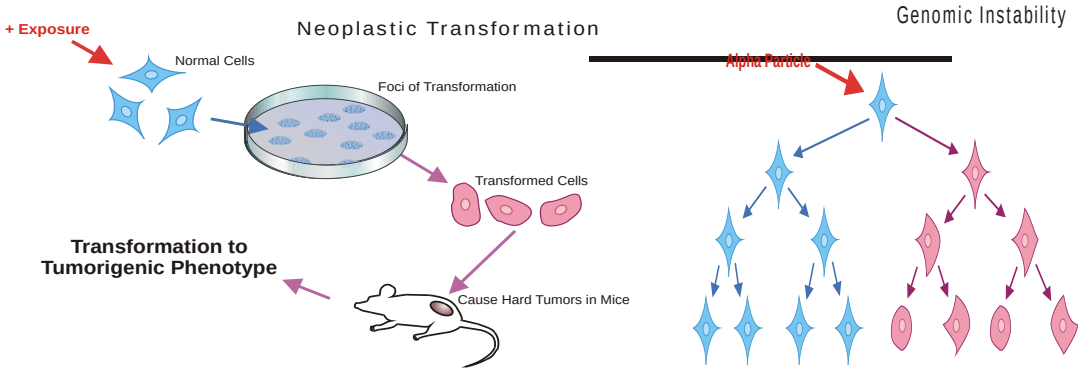
Short-Term Carcinogenicity Tests *In Vitro*:

Relative Comparison of DU, Nickel, and Alpha Particles





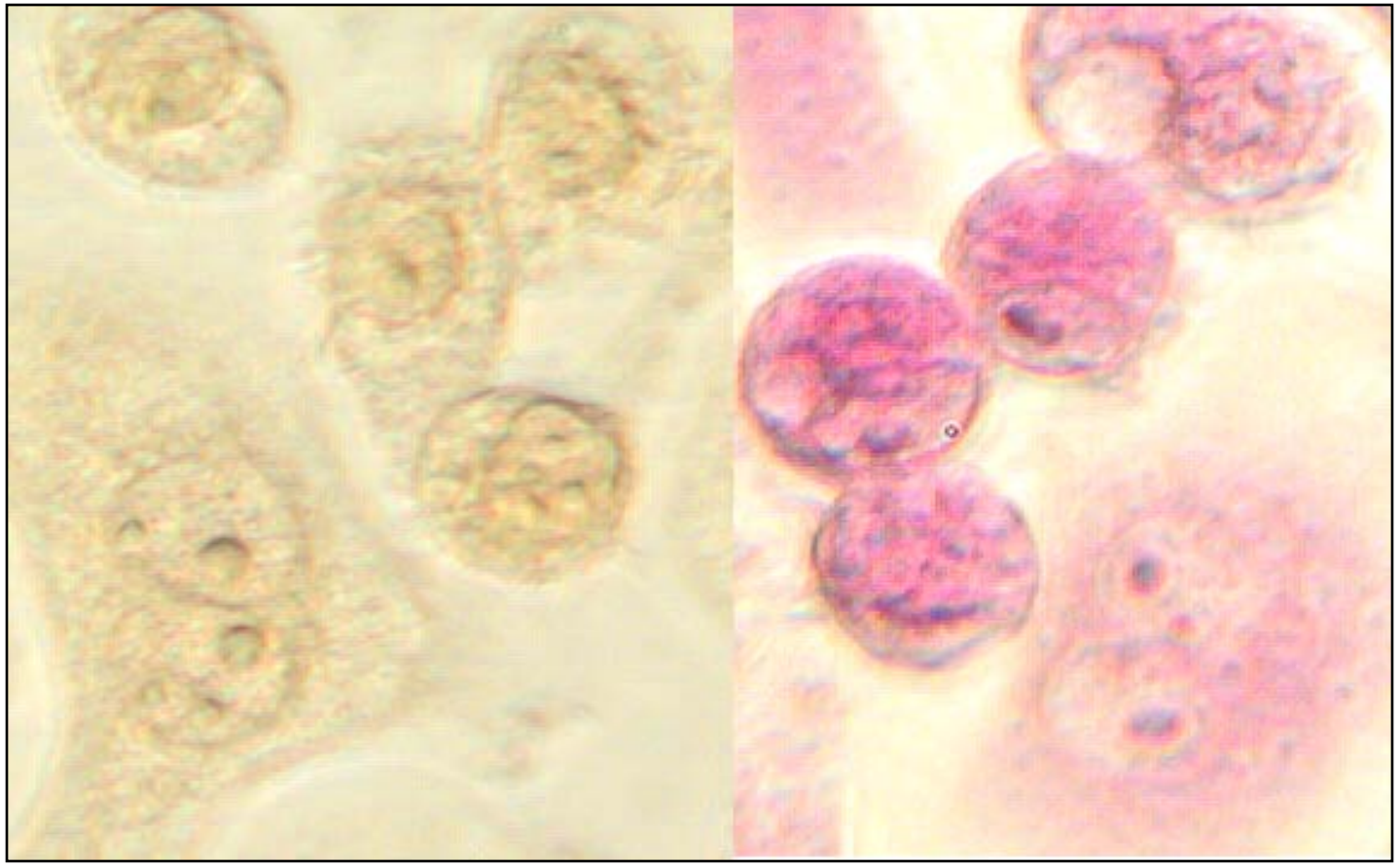




Miller, *et al, Environmental Health Perspectives*, Vol. 106, 1998; Miller, *et al, Carcinogenesis*, Vol. 22, 2001. Miller, *Reviews on Environmental Health,* Vol 22, 75-94, 2007

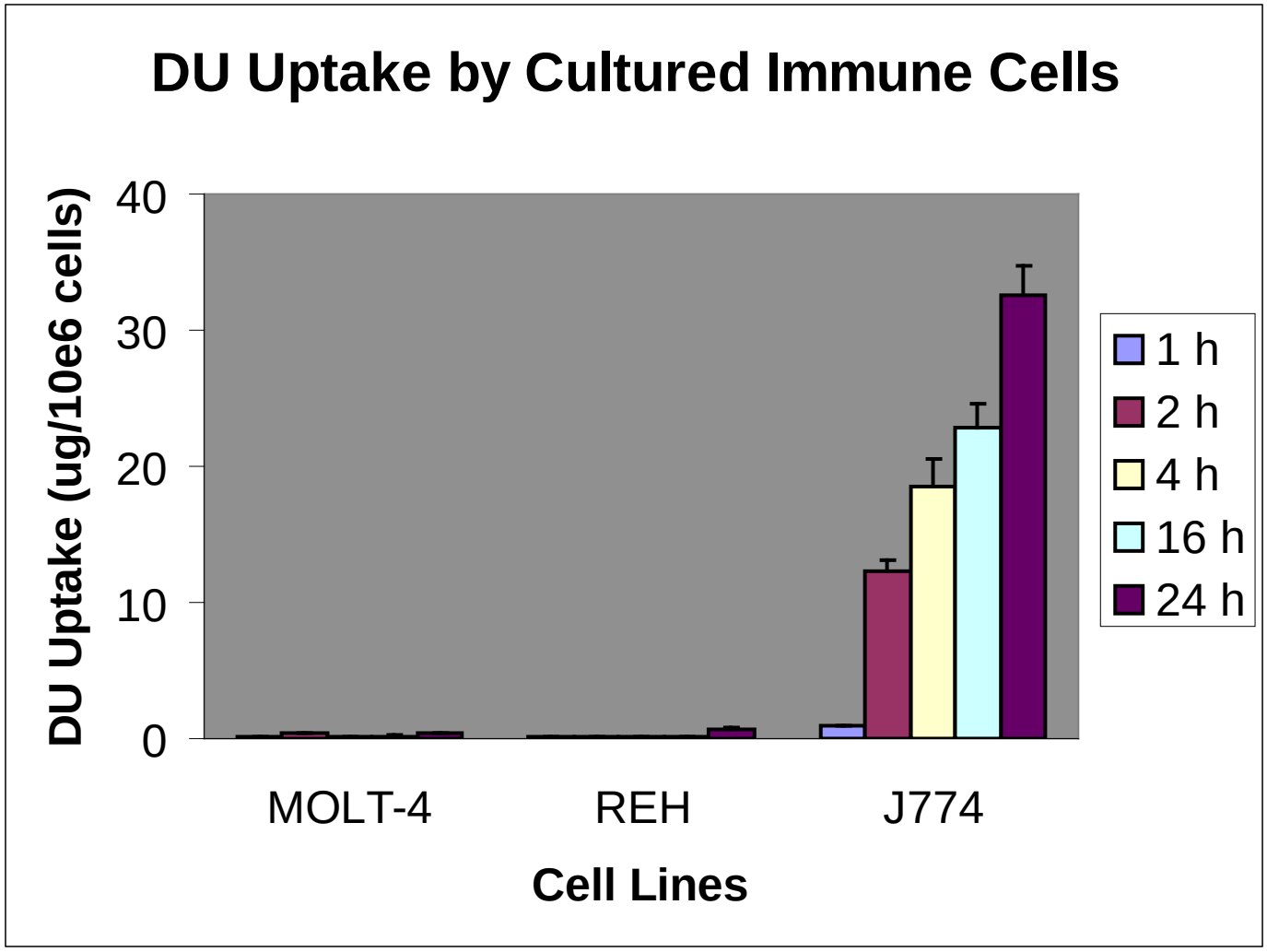
Molecular and Cellular Effects of DU

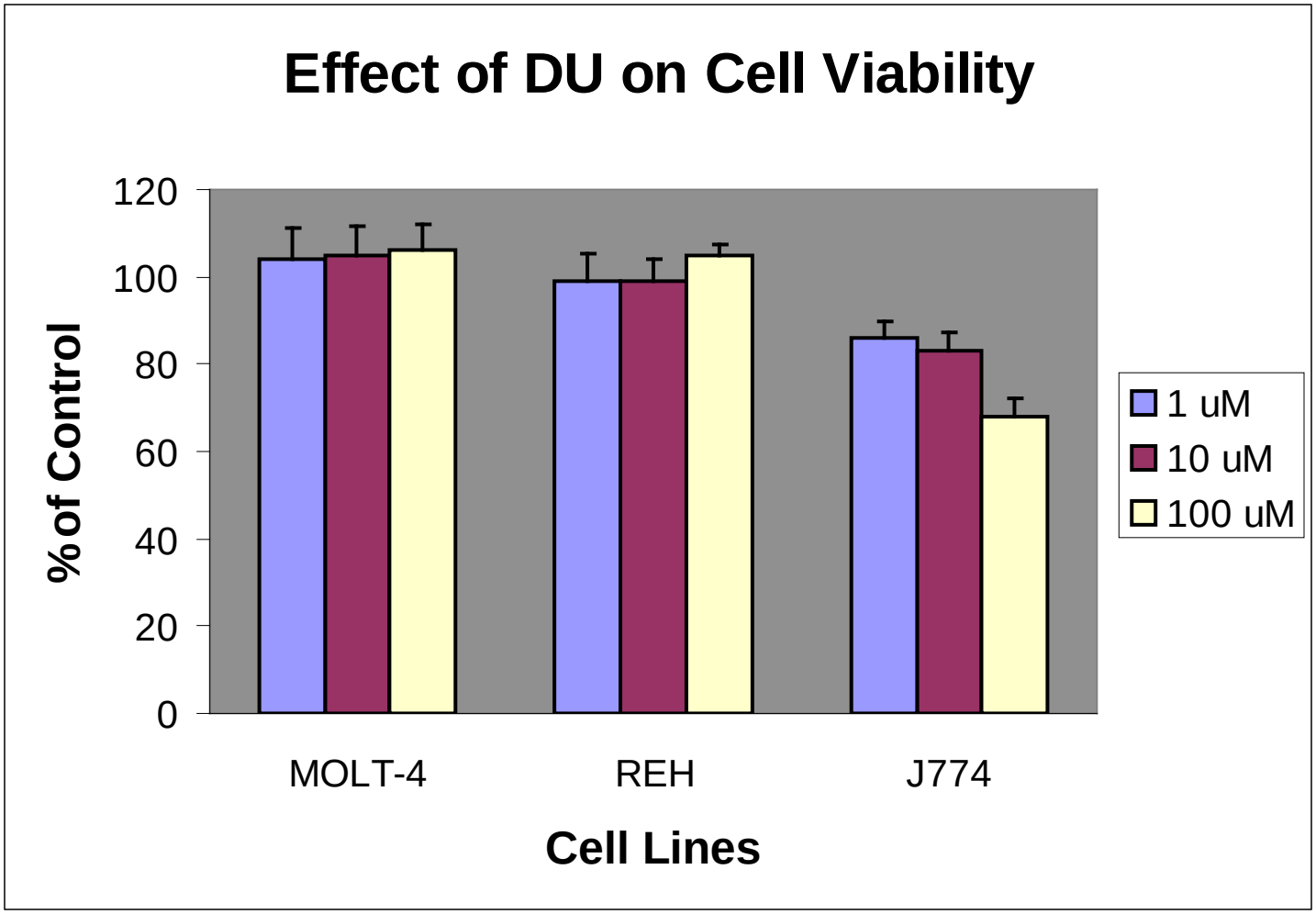
PADAP Staining of DU-treated J774 Cells



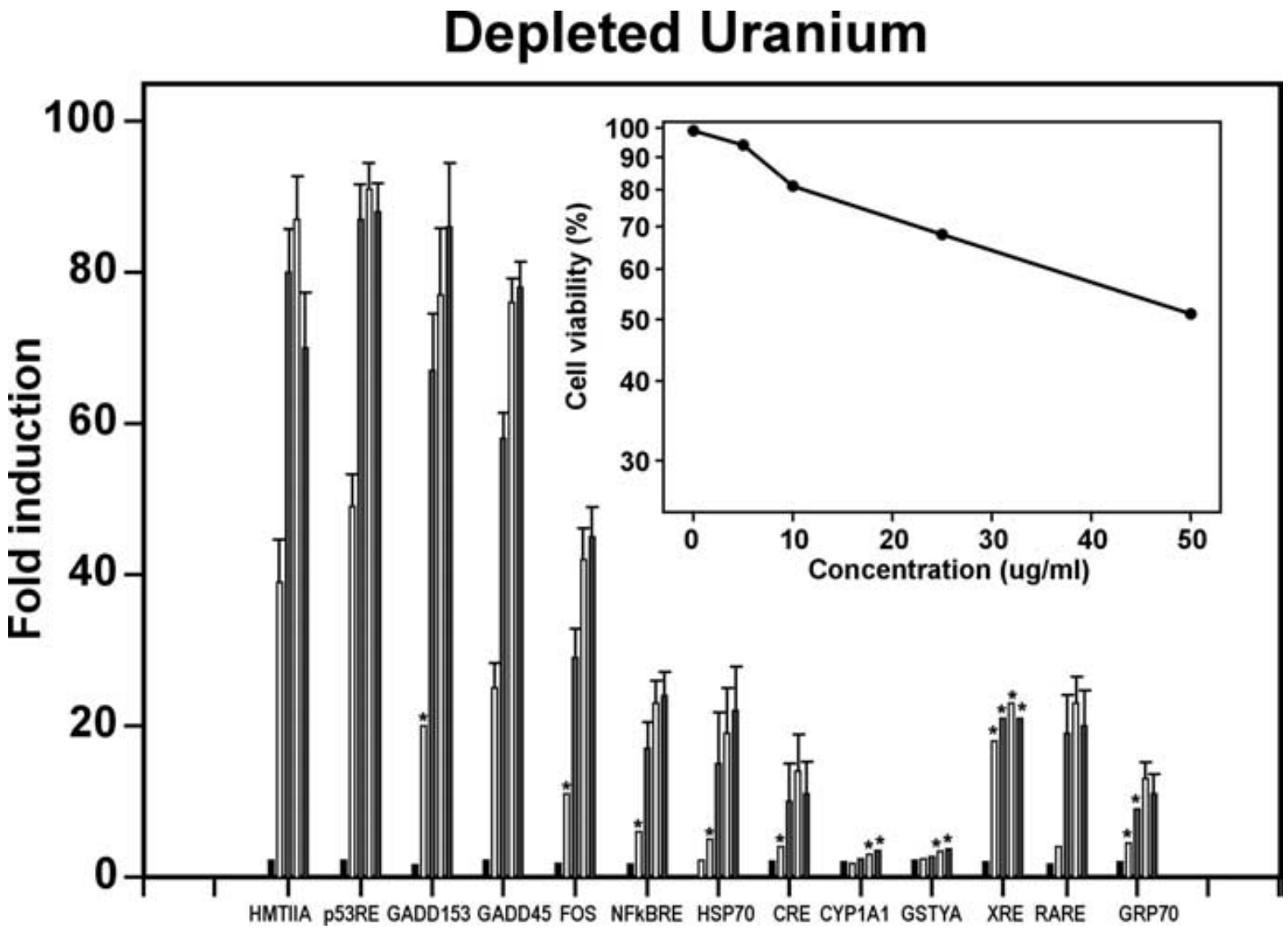
Without DU

With DU


DU Uptake by Cultured Immune Cells
DU Uptake (ug/10e6 cells)
40
30
20
10
0
MOLT-4
REH
J774
Cell Lines
1 h
2 h
4 h
16 h
24 h

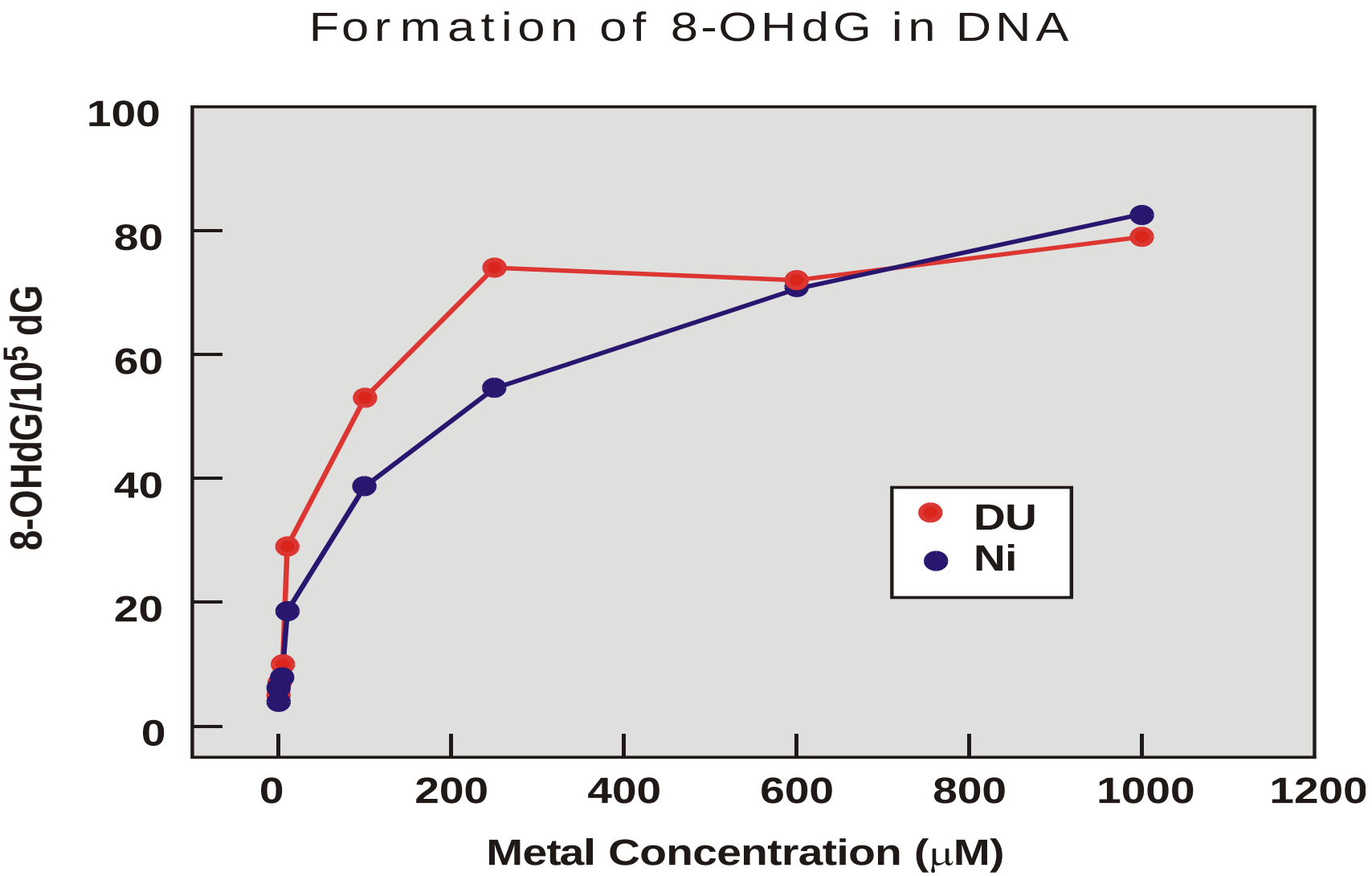

Effect of DU on Cell Viability
120
100
80
60
40
20
0
% of Control
MOLT-4
REH
J774
Cell Lines
1 uM
10 uM
100 uM

DU Effects on Gene Expression



Miller et al. 2004 *Molecular and Cellular Biology*

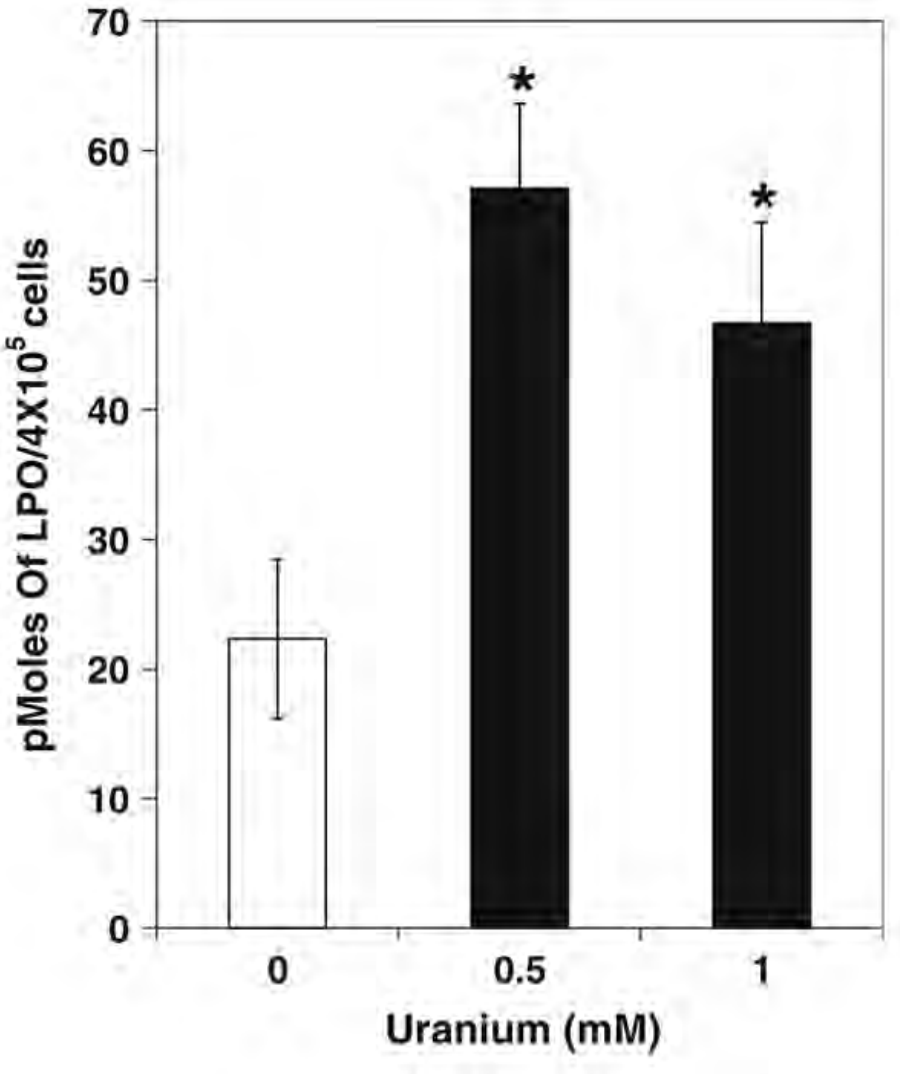
Role of Oxidative DNA Damage in Human Cells



Miller AC, et al, 2002, *J Inorg Biochem*

DU Induces Oxidative Stress

Rat lung epithelial cells
Reduction in GSH
Reduction in SOD



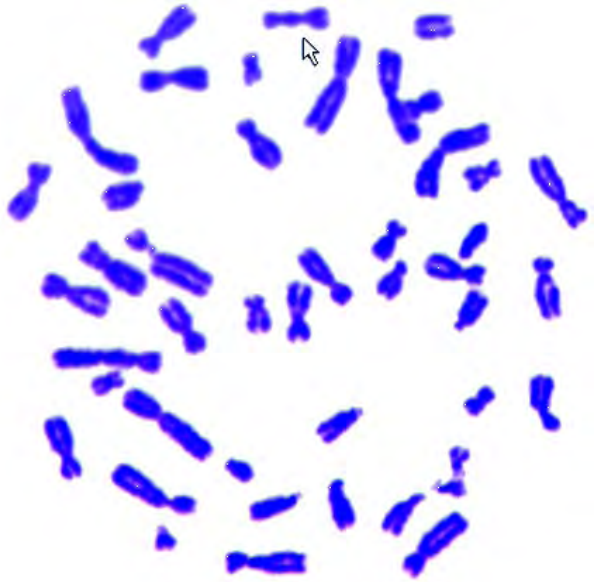
Periyakaruppan et al., 2007, *Archives Toxicology*

Radiation versus Chemical Effects

Does DU Cause Radiation Specific Damage?

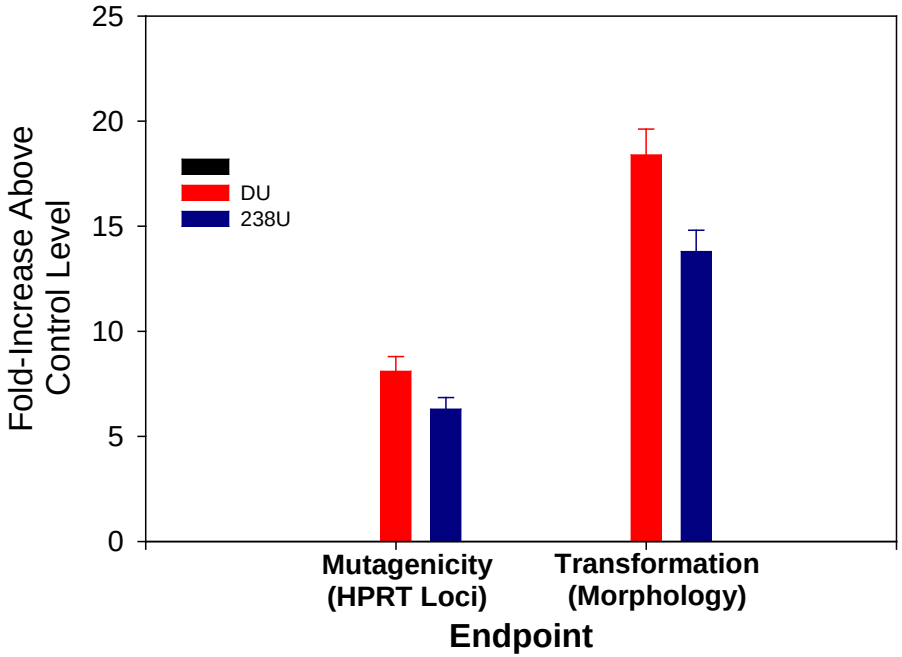
Radiation Effects of DU: *In vitro* studies
Two Approaches

1. Radiation-specific Damage - Dicentric Chromosomes



2. Uranium Isotope Comparison at Equal Chemical Concentration

Uranium Isotopes:	**Rad Activity**	**Chem Tox**
DU	**0.43**	**1.0**
^{238}U	**0.33**	**1.0**

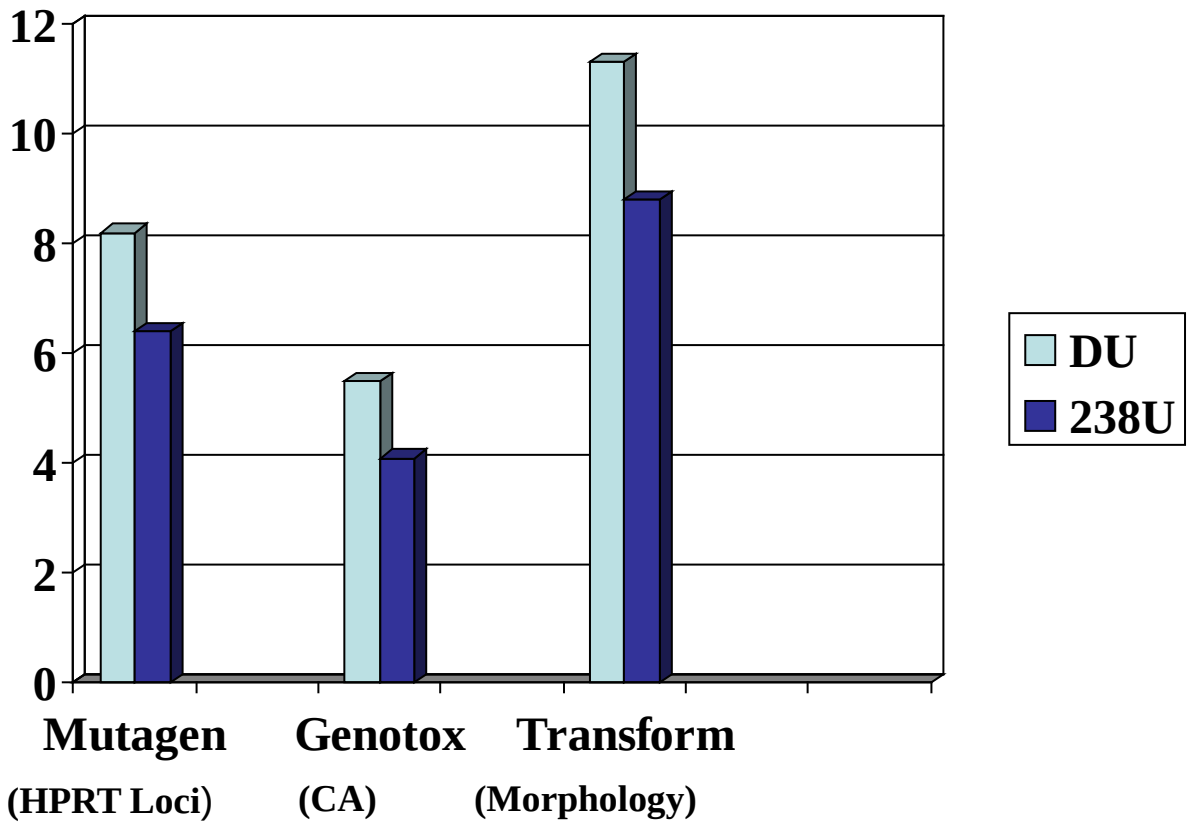


Miller, *et al., Radiat Prot Dosimetry*, 99(1-4):275-8, 2002
Miller *et al., Radiation Measurements*, 42:6-7:1090, 2007.

Radiation Specific Effects *in Vitro*:

Heavy Metal Mutagenicity, Genotoxicity Neoplastic Transformation:

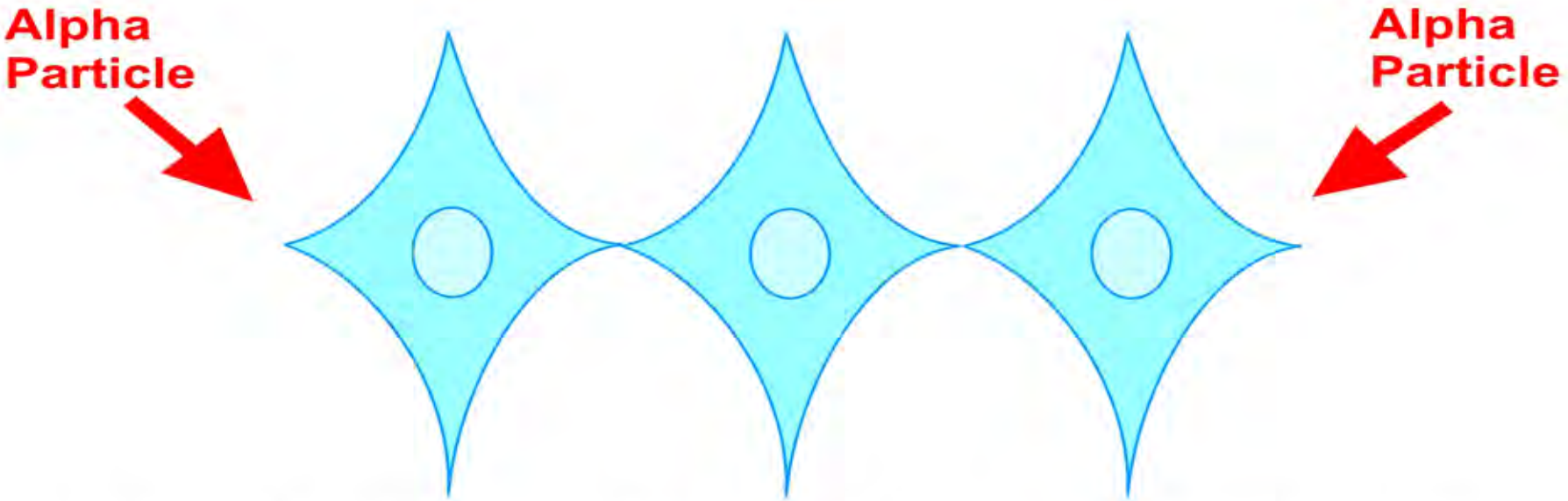
Comparison of DU and ^{238}U at Equal Concentrations



Uranium Isotopes:	Specific Activity
^{235}U	2.2
DU	0.43
^{238}U	0.33

Miller, *et al, Environmental Health Persp*, Vol. 106, 1998
Miller, *et al, Carcinogenesis*, Vol. 22, 2001.
Unpublished data.

Third Approach:
Mimic Radiation Dose from DU
Radiation Dose Measurement
"Microdosimetry"

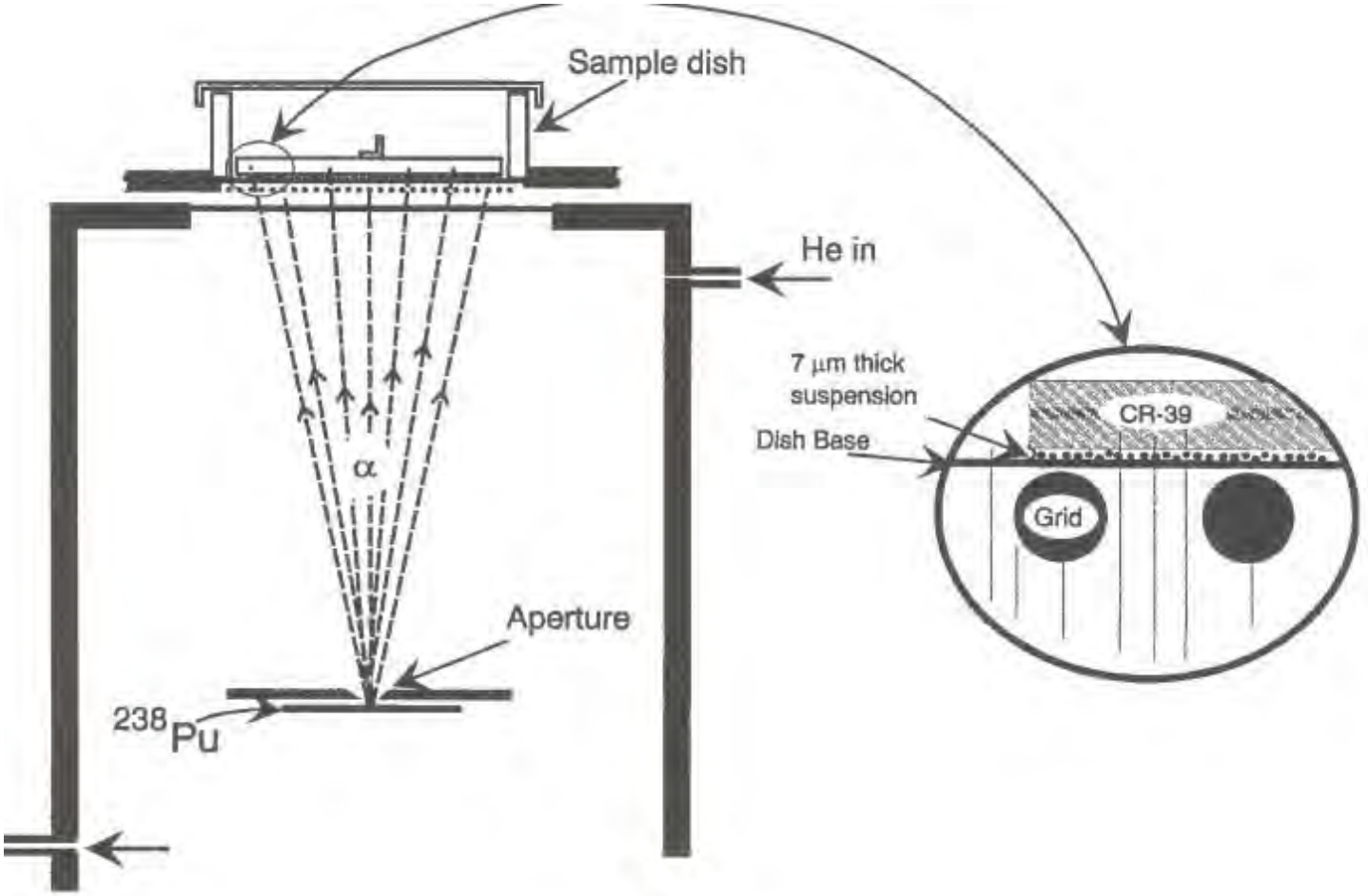


≤ 1% cells hit; approximately 18 cGy (soluble DU)

≤ 7% cells hit; approximately 22 cGy (insoluble DU)

≤ 21% cells hit; approximately 24 cGy (insoluble DU)

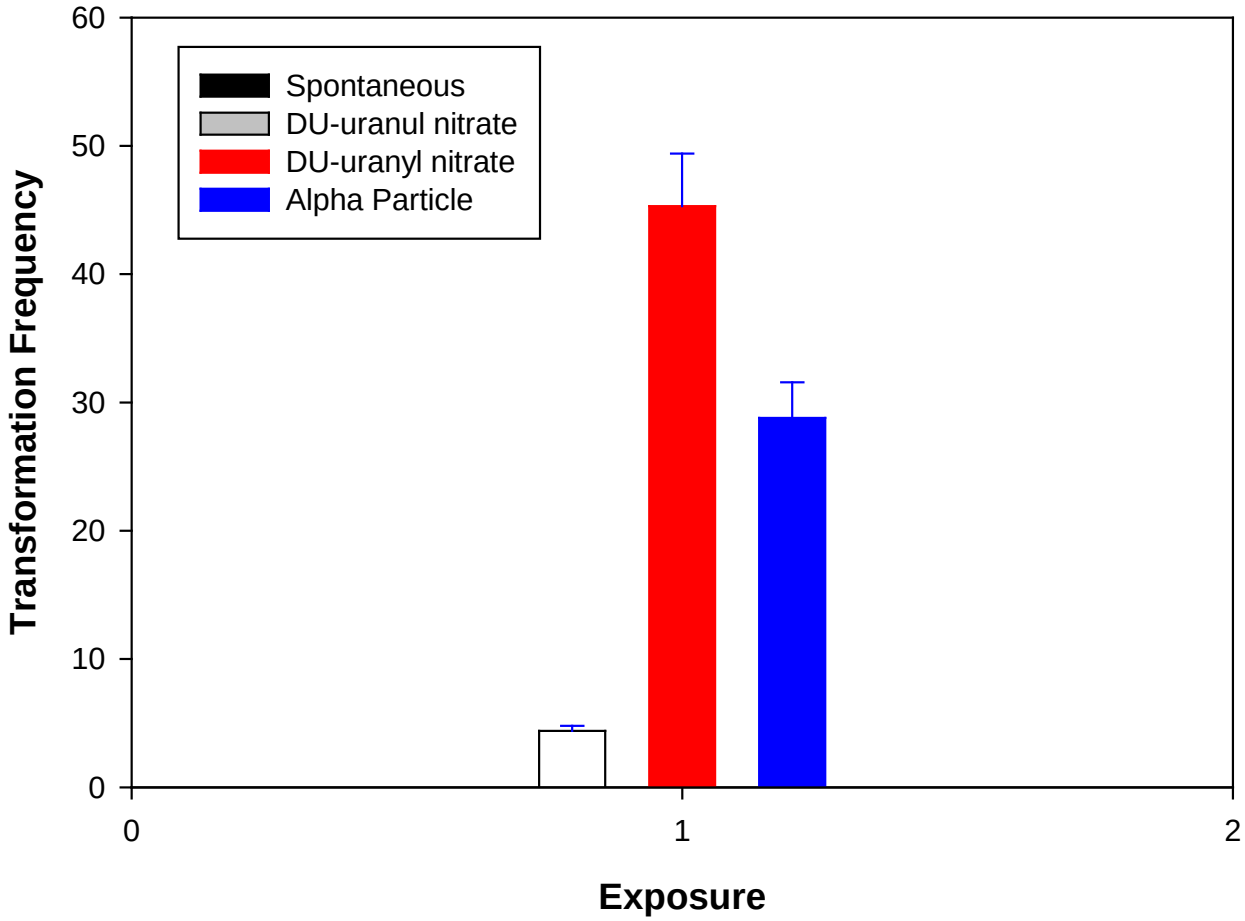
Third Approach:
Schematic Diagram of Alpha-Particle Irradiator:
Shielding Effect of Interposing Grid Between Source and Cells



Medical Research Council U.K., Radiation and Genome Stability Unit ; Harwell, Oxfordshire, U.K.
Lorimore, Kadhim, Goodhead, Wright, *PNAS,* Vol 95: 5730-33, 1998.

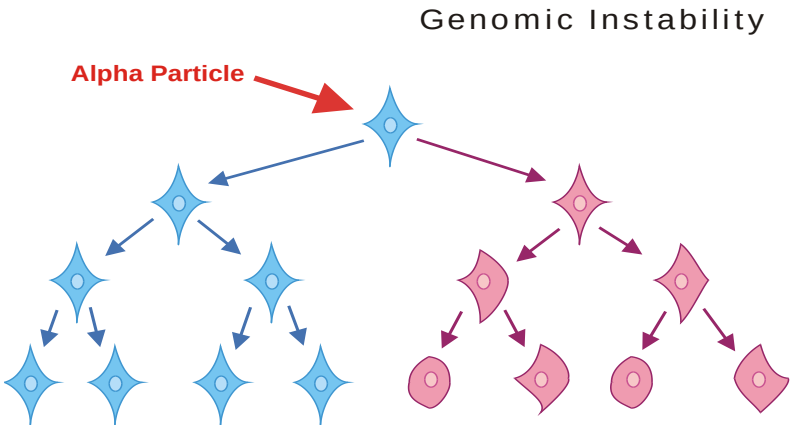
Comparison of Alpha Particle Exposure from DU To Alpha Particles from Alpha Source

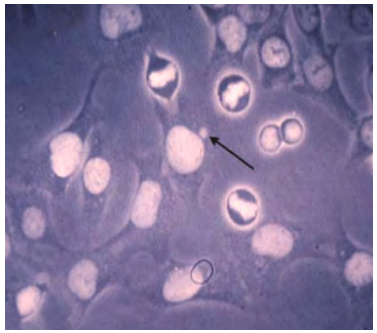
17% Cell Nuclei Traversed by Alpha Particle from Either Source



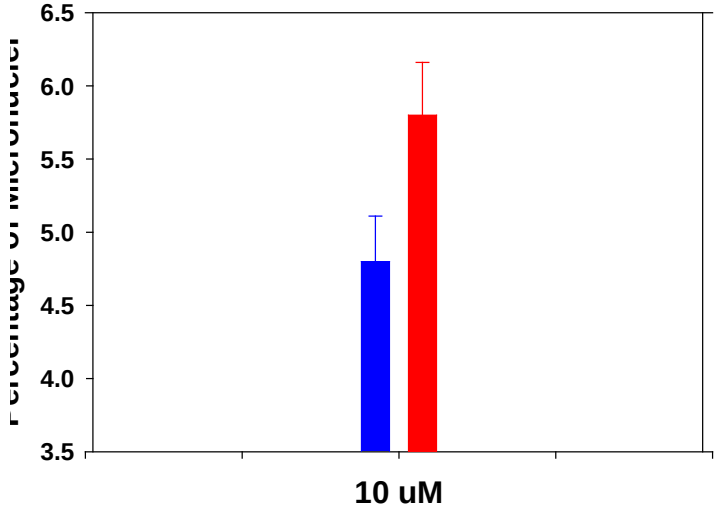
Third Example of DU Radiation Effects: Genomic Instability

Uranium Isotopes:	Rad Activity	Chem Tox
DU	0.43	1.0
^{238}U	0.33	1.0





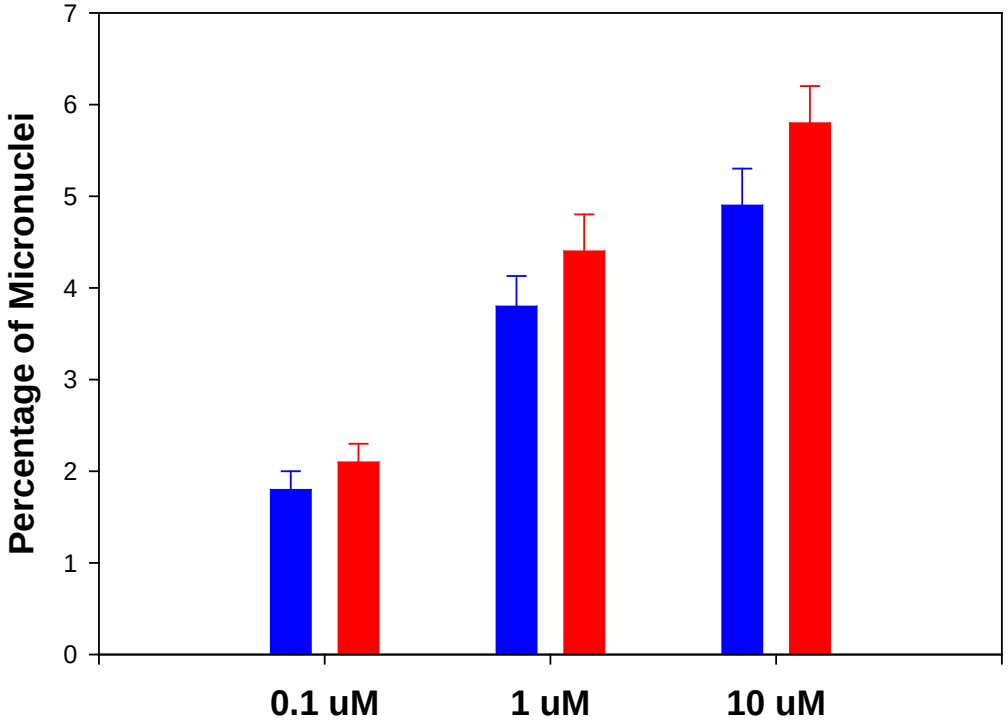
Number of Clones Tested (20)



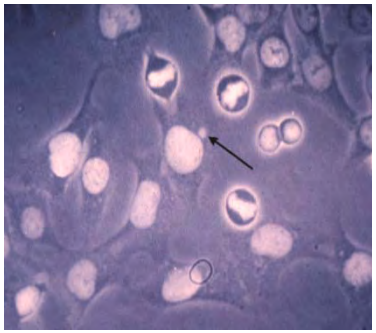
Special thanks to Gwen Watson, MRC UK

Micronuclei as Endpoint of Genomic Instability Due to Radiation Effect

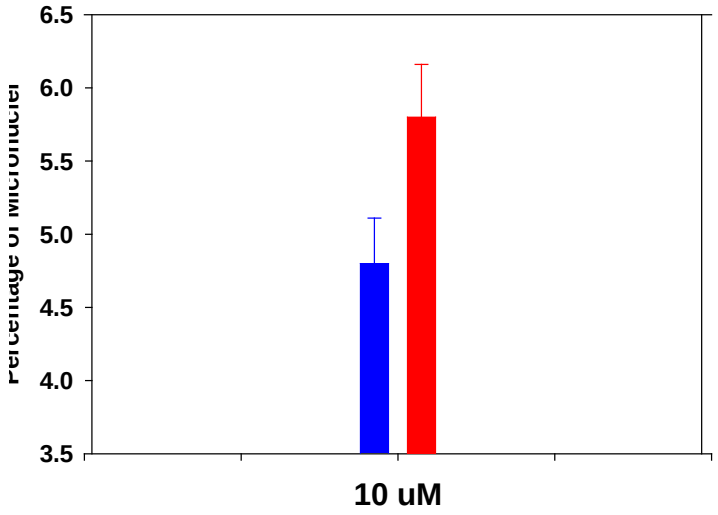
Number of Clones Tested (5-7)



Uranium Isotopes:	Rad Activity	Chem Tox
DU	0.43	1.0
^{238}U	0.33	1.0



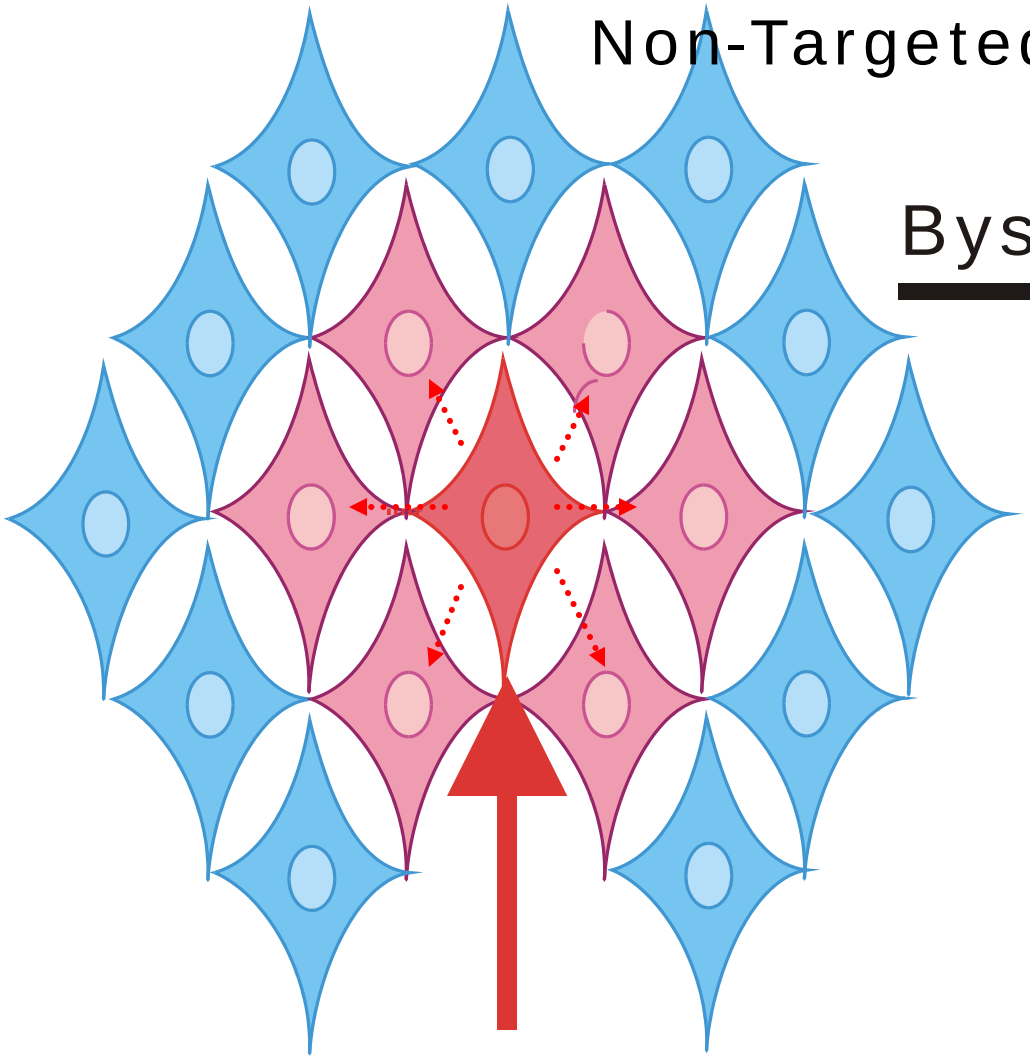
Number of Clones Tested (20)



Special thanks to Gwen Watson, MRC UK

Non-Targeted Radiation Effect

Bystander Effects

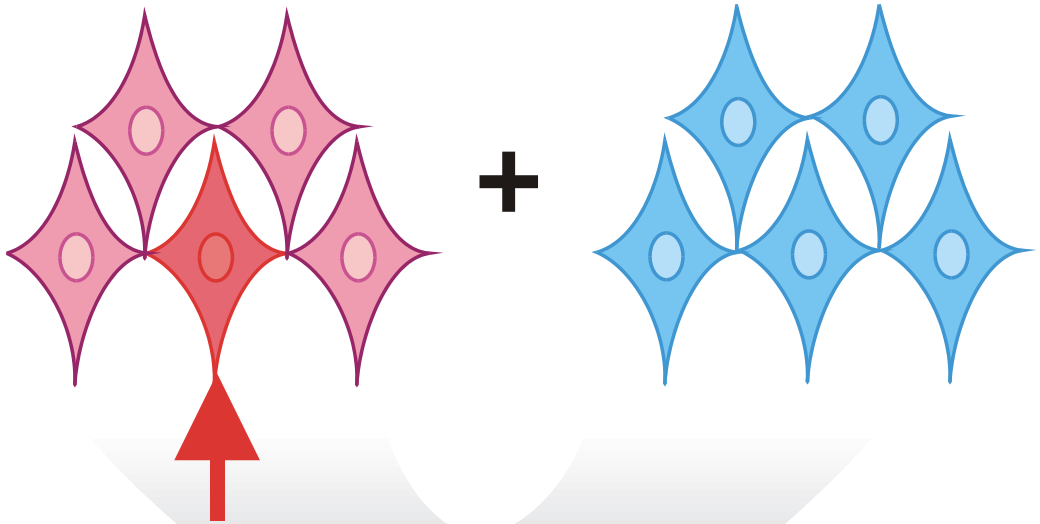


Alpha Particle

1 cell hit but > 2 cells affected

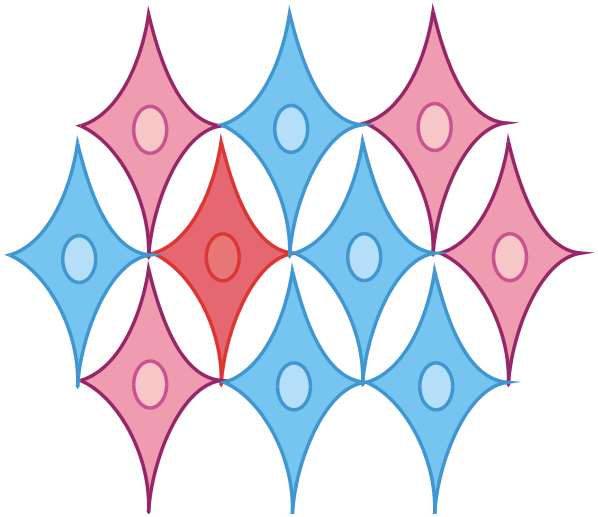
Bystander effects have been reported for a variety of endpoints using single-cell systems

- ✓ **Mutation induction**
- ✓ ***In-vitro* oncogenic transformation**
- ✓ **Changes in gene expression**
- ✓ **Altered cell growth**
- ✓ **Sister-chromatid exchanges**
- ✓ **Cell killing (mitotic and apoptotic)**
- ✓ **Micronucleus induction**



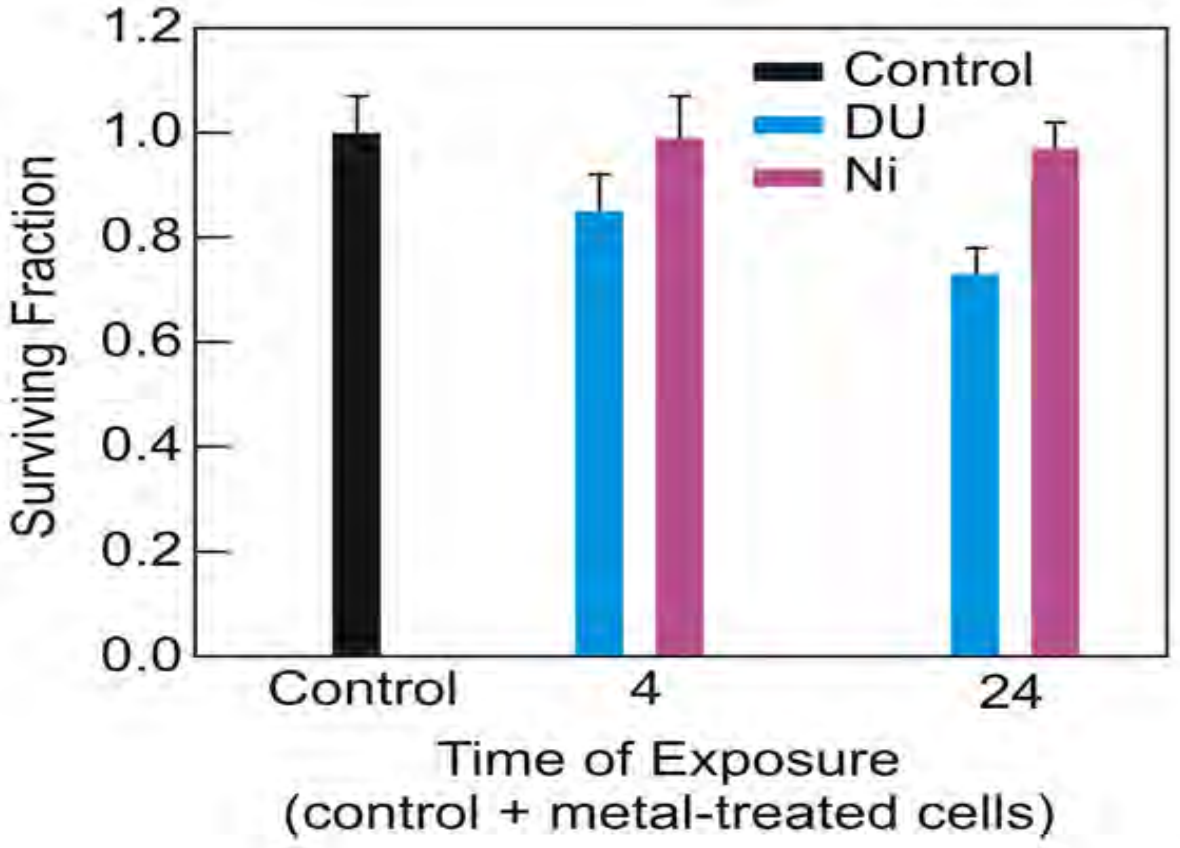
DU Exposure

Rinse Cells

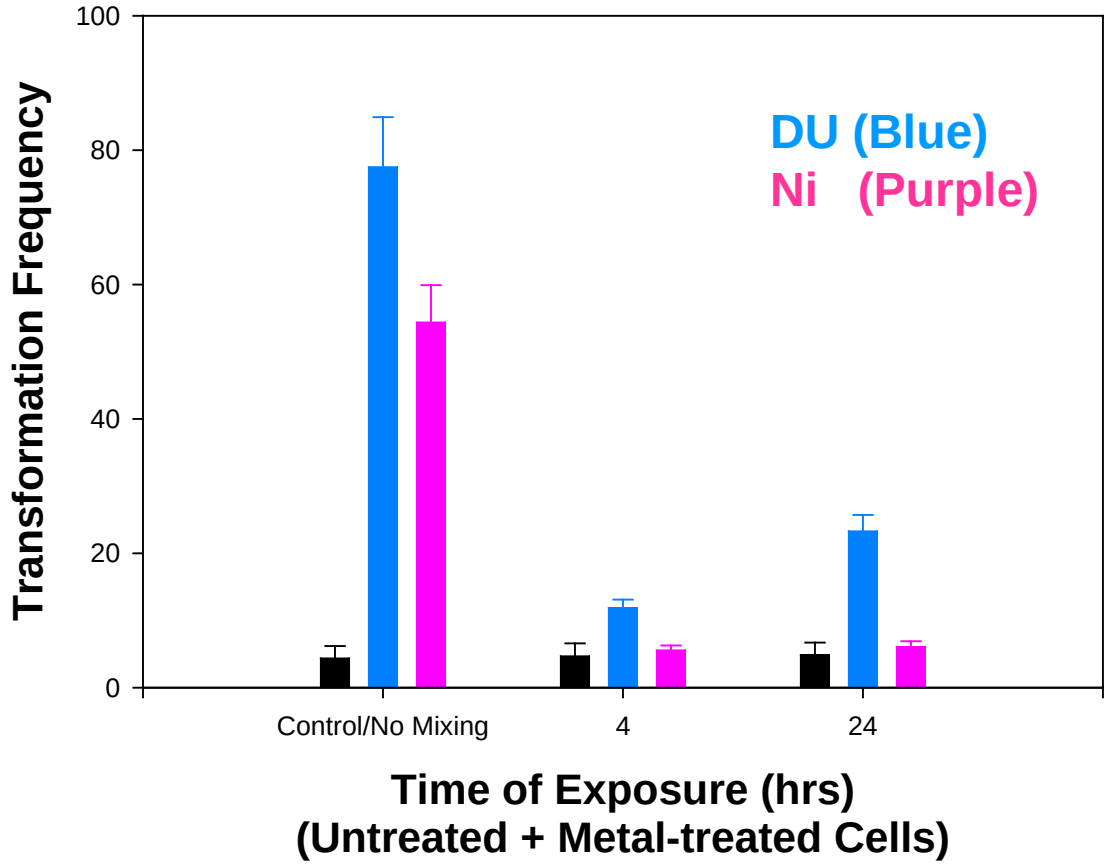


Mixed Populations Analysis

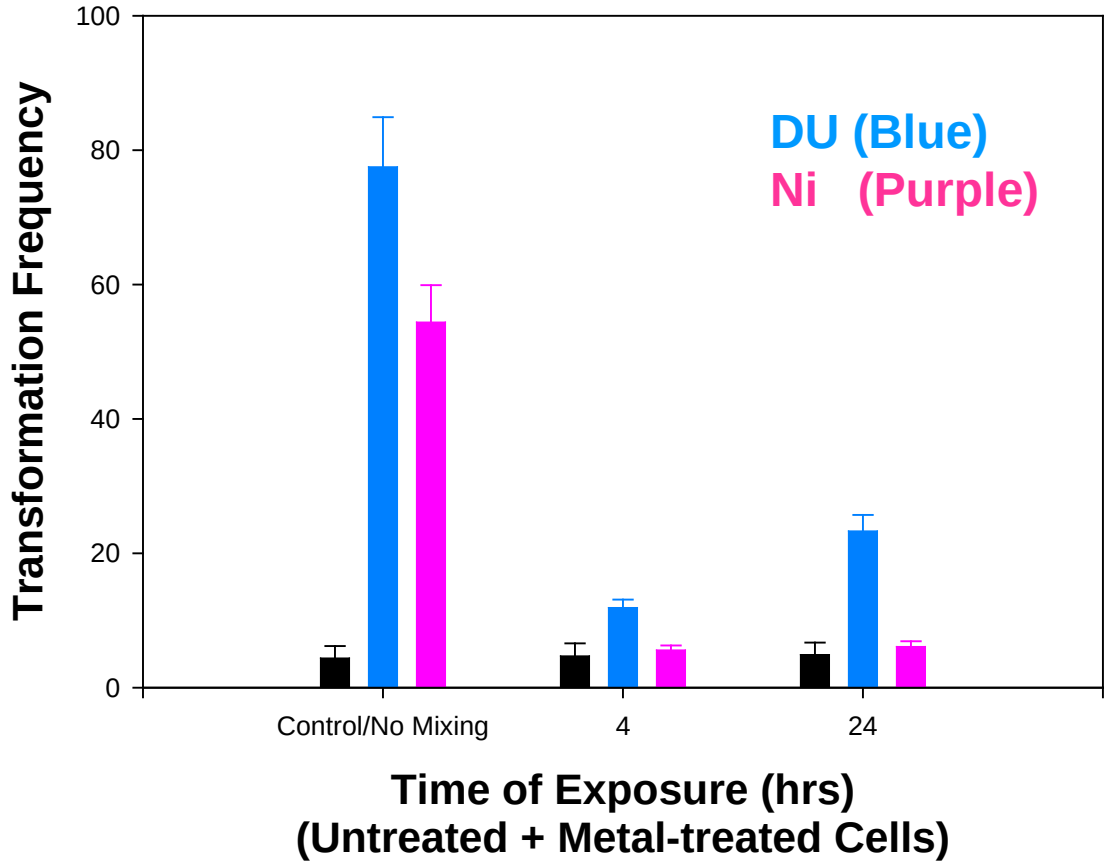
Surviving Fraction of Bystander HOS Cells Co-cultured with HOS Cells Exposed to Heavy Metal



Transformation Frequency of Bystander HOS Cells Co-cultured with HOS Cells Exposed to Heavy Metal

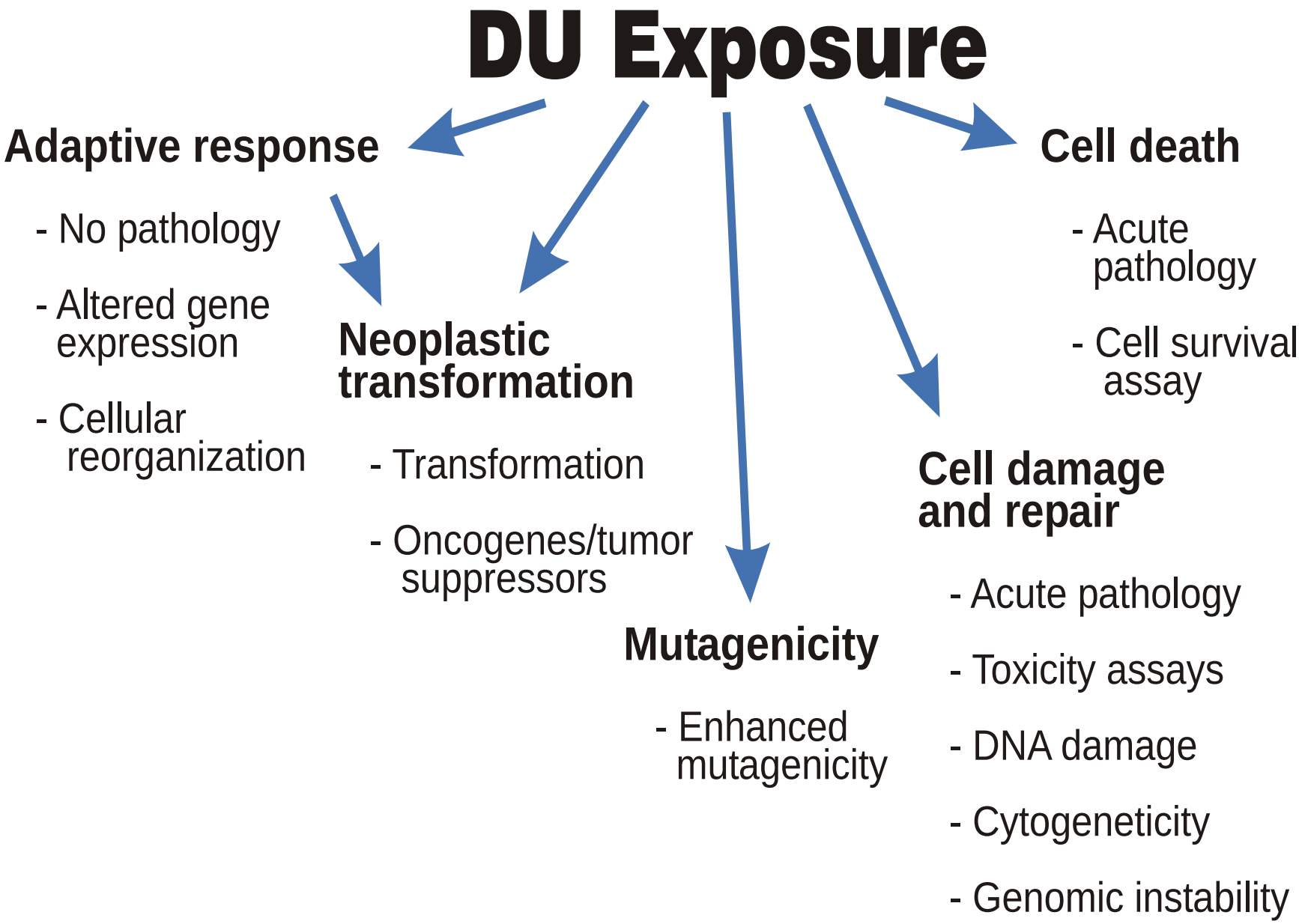


Transformation Frequency of Bystander HOS Cells Co-cultured with HOS Cells Exposed to Heavy Metal



Bystander Effects

So yes, beware thy neighbor


DU Exposure
Adaptive response
- No pathology
- Altered gene expression
- Cellular reorganization
Neoplastic transformation
- Transformation
- Oncogenes/tumor suppressors
Mutagenicity
- Enhanced mutagenicity
Cell damage and repair
- Acute pathology
- Toxicity assays
- DNA damage
- Cytogeneticity
- Genomic instability
Cell death
- Acute pathology
- Cell survival assay

Other studies *in Vitro:*

1. **DU induces kidney cell toxicity *in vitro*.**.Goldman et al, 2006 , “Nephrotoxicity of uranyl acetate: effect on rat kidney brush border membrane vesicles”. *Archives Toxicology* Jul;80(7):387-93

2. **DU induces neurotoxcity *in vitro*.** Aschner et al., 2006, “Neurotoxicity of depleted uranium: reasons for increased concern”. *Biol Trace Elem Res.,* Apr;110(1):1-17.

3. **DU induces immune toxicity in macrophages.** Wan B et al, 2006, “In vitro immune toxicity of depleted uranium: effects on murine macrophages, CD4+ T cells, and gene expression profiles. *Environ Health Perspect.* Jan;114(1):85-91.

4. **DU cytotoxicity is associated with mitochondrial/lysosomal toxicity by the reduced biological metabolites and ROS.** Pourahmad, et al. 2006, *Environmental Toxicol*, Aug;21(4):349-54

5. **DU is absorbed by intestinal cells but is not toxic.** Dublineau I, et al, 2006, Toxicology. 227(3):227-39.

6. **Suppression of DU-Induced Transformation Can be Achieved Pharmacologically Using Phenyl Fatty Acids,** Miller AC, et al, 2001, “Suppression of DU-Induced Neoplastic Transformation. Radiat Res. 2001 Jan;155(1 Pt 2):163-170.

Conclusions *in Vitro:*

1. **DU induces neoplastic transformation, mutagenicity, and genotoxicity *in vitro.***

2. **DU is involved in uranium-induced genomic instability.**

3. **Alpha particles similar in energy and distribution to those resulting from cellular uranium exposure to DU are sufficient to transform cells.**

4. **Radiation bystander effects are involved in uranium-induced neoplastic transformation and genomic instability.**

Acknowledgements

AFRRI – My Lab
Stuart Cohen
Rafael Rivas
Karvi Miller

AFRRI
Mike Stewart
Chris Lissner
John Gilstad
Terry Pellmar
John Kalinich
Dave McClain
Bill Blakely
David Livengood
PGS Prasanna
Henry Gerstenberg
John Ejnik
Ted St. John
Shelly Hodge
Christy Emond
Vernieda Vergara
Tom Dalton
Jessica Kordell
Danny Beltran
Tim Whittaker
Jiaquan Xu
Nalaja Marcus
Kia Brooks

Columbia University
Tom Hei
David Brenner
Steve Marino
Aparajita Dutta
Catherine Mitchell
Jo Tsakok
Satin Sawant
Steve Mitchell

University Of Paris
Paul Lison
Robert Merlot
Catherine Bonait-Pellie
Jean Michel
Lillian Crepin

French Institute
Nuclear Security
Francois Paquet
Valerie Chavel
Pascal Houpert
Henri Metivier

NIH/NCI
Hannah Hsu
Tom Wang
Lei Luo
Natalie Page
John Chen

UK Medical Research Council
Munira Kadhim
Dudley Goodhead
Mark Hill
Rona Anderson
Hooshang Nikjoo

University of Southern Maine
John Wise Hong Xie
Kelly Joyce

Memorial Sloan Kettering Cancer Center
John Humm

This work was supported by NIH Grant P41-EB002033; AFRRI/USUHS Intramural Project RAB5AA, Defense Threat Reduction Agency Grant G1B2BJ, G1B2EM, DMRDP